# NOVEL MULTICOMPONENT ORGANIC-INORGANIC WHEY PROTEIN ISOLATE (WPI)/GELATIN/CALCIUM PHOSPHATE-HYDROGEL COMPOSITES FOR BONE TISSUE ENGINEERING

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## Introduction

The present work focuses on the development of novel multicomponent organic-inorganic hydrogel composites for bone tissue engineering. A combination of the organic components commonly used in food industry, namely whey protein isolate (WPI) and gelatin from bovine skin, as well as inorganic material commonly used as a major component of hydraulic bone cements, namely α-TCP in various concentrations (0-70 wt.%) was proposed.

## Materials and Methods

WPI (BiPro, Davisco Foods International Inc., USA), 97.7% protein, 75% beta-lactoglobulin by dry mass, and gelatin from bovine skin type B (Sigma-Aldrich, UK) were used. α-TCP was produced by a wet chemical method. To produce composites, 40 wt./vol.% WPI solution was mixed with gelatin powder (20 wt%) in ultrasonic bath (40°C) for 30 min. Warm WPI/gelatin solution was mixed with α-TCP powder in 2 mL Eppendorf tubes for 30 s. Closed tubes were immersed in cold (-20°C) ethanol to induce fast gelation. After 2 minutes, tubes were immediately heated at 100 °C for 5 min to induce WPI thermal crosslinking, then autoclaved (121°C for 30 min). α-TCP concentrations of 20, 30, 40, 50, 60, and 70 wt% were compared. Human osteoblast-like MG-63 cells (Sigma Aldrich, USA) were seeded on materials in a concentration of $10.5 \times 10^3$ $cm^{-2}$ in 1 ml of culture media. Metabolic activity was assessed by the MTS assay.

## Results and Discussion

α-TCP underwent incomplete transformation to calcium-deficient hydroxyapatite (CDHA). The values of Young's modulus and the stresses corresponding to compression of a sample by 50% increased almost linearly with increasing concentration of ceramic phase (FIG. 1). Microcomputer tomography showed inhomogeneous distribution of the calcium phosphate (CaP) phase in the resulting composites (FIG. 2). In vitro studies revealed that the composites support adhesion, spreading, and proliferation of MG-63 cells (FIG. 3).

## Conclusions

The composites show potential as materials for tissue regeneration and can potentially be processed into 3D scaffolds using a low temperature 3D-printing technique.

## Acknowledgment

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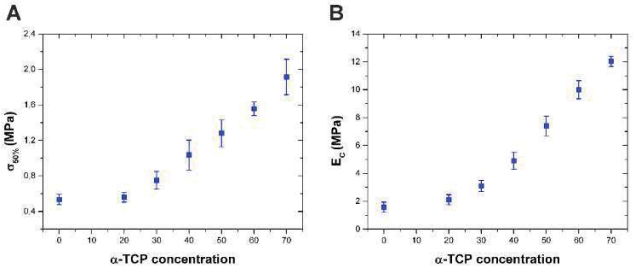


FIG. 1. Compressive strength at 50% strain σ50% (A) and compressive modulus EC (B) of the WPI/gelatin/CaP hydrogel composites.

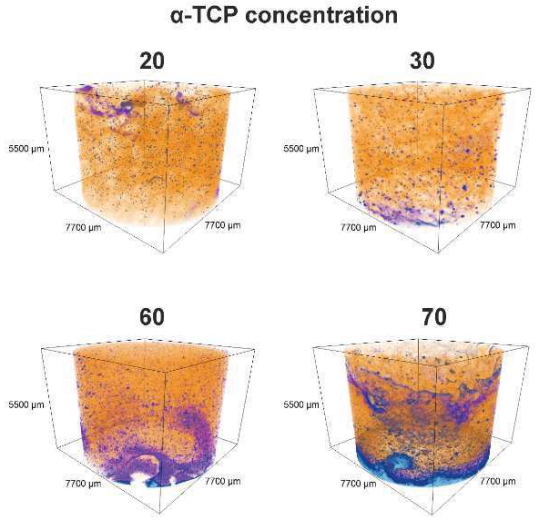


FIG. 2. µCT analysis of the WPI/gelatin/CaP hydrogels - 3D rendering.

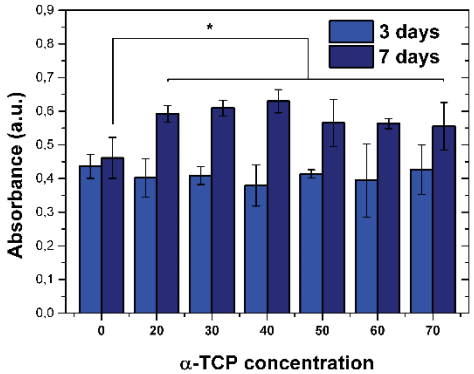


FIG. 3. Metabolic activity of MG-63 cells cultured for 3 and 7 d in direct contact with composites. Statistically significant differences ($p < 0.05$) relative to the hydrogel unmodified with CaP are indicated by asterisk * (differences detected only at 7 d).