DOLOMITE AND CALCITE ENHANCEMENT OF WHEY PROTEIN ISOLATE HYDROGELS

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Introduction

Calcite, the thermodynamically most stable polymorph of calcium carbonate ($CaCO_3$), has successfully been used to promote bone regeneration [1]. Dolomite ($CaMg(CO_3)_2$), is a form of magnesium calcite, which is used in the building industry and is available in large amounts in Poland. Mg promotes bone-forming cell proliferation as a component of calcium phosphate (CaP) [2]. We hypothesized that Mg incorporation into $CaCO_3$ would also positively influence cell behaviour, and that addition of dolomite and calcite to hydrogels, (highly hydrated three-dimensional polymer networks) would improve cell proliferation. Addition of preformed inorganic particles to hydrogels is a common mineralization strategy [3]. In this study, dolomite and calcite particles were added to hydrogels of whey protein isolate (WPI), an inexpensive by-product from the dairy industry, and has displayed positive biological effects in our previous work [4]. WPI hydrogels can be formed by heat sterilization, e.g. autoclaving.

Materials and Methods

Synthetic calcite was prepared as described previously [5]. Dolomite was obtained from the Ołdrzychowice region of Lower Silesia, Poland, as described previously [6]. Composites were produced by the heat-induced gelation of 50% (w/v) WPI solution, with 30% (w/v) calcite or dolomite particles added (denoted hereafter as WPI-calcite and WPI-dolomite, respectively). 1 ml composites were formed in 2 ml Eppendorf tubes.

Composites were investigated by assessing particle distribution (Micro-CT imaging) and cytocompatibility. 10,000 MG63 cells were seeded on WPI-calcite and WPI-dolomite (n=5) and polystyrene (n=4). Proliferation after 1, 4 and 7 days was assessed using the fluorescent PrestoBlue assay. Fluorescence microscopy after DAPI staining was also performed.

Results and Discussion

Micro-CT analysis (Bruker) suggested good cross-sectional distribution of both calcite and dolomite particles within hydrogel-particle composites (FIG. 1).

MG63 cells proliferated on both WPI-calcite and WPI-dolomite, though to a lesser extent than on polystyrene (FIG. 2).

Cells showed a well-spread morphology (FIG. 3). Proliferation increased over 7 days. No significant differences were observed between WPI-calcite and WPI-dolomite. Further work will focus on increased physicochemical characterization of composites and cell biological characterization, possibly with primary cells instead of a cell line.

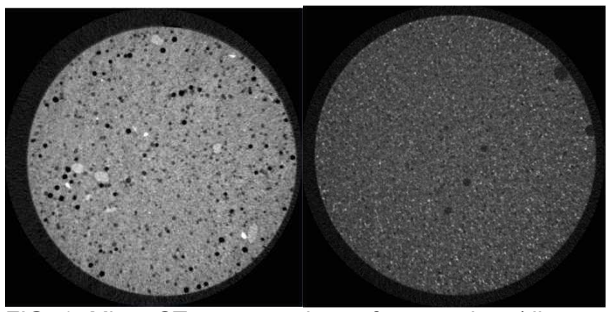

FIG. 1. Micro-CT cross-sections of composites (diameter 8 mm). Left: WPI-calcite. Right: WPI-dolomite. White dots indicate the presence of mineral. Black dots indicate cavities.

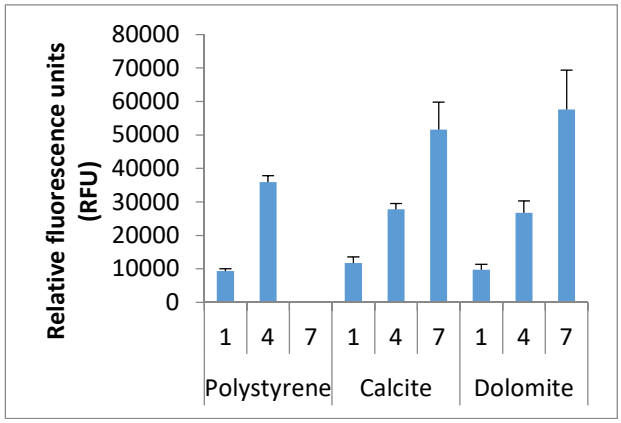

FIG. 2. Proliferation of MG63 cells over 7 days on Polystyrene, WPI-calcite and WPI-dolomite. Error bars are representative of standard deviation.

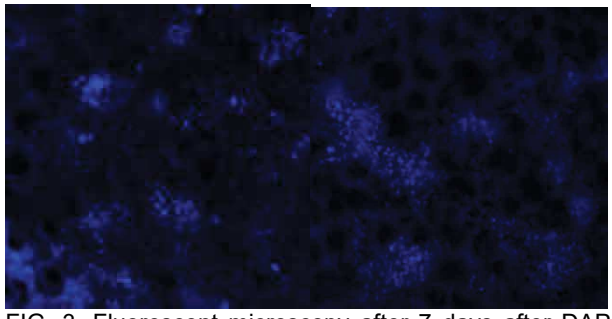

FIG. 3. Fluorescent microscopy after 7 days after DAPI staining. Magnification x5. Left: WPI-calcite. Right: WPI-dolomite.

Conclusions

WPI-calcite and WPI-dolomite composites both displayed cytocompatibility and supported MG63 cell adhesion and proliferation. Thus, both composites appear to be promising materials for bone tissue regeneration.

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