BIOMIMETICALLY MINERALIZED GELATIN HYDROGELS PRODUCED BY NOVEL BETA-RADIATION CROSSLINKING

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Introduction

Hydrogels have numerous potential advantages in tissue engineering. One of these is the ease of incorporation of bioactive substances such as enzymes. Gelatin is a promising biomaterial, being inexpensive, biocompatible, non-immunogenic and biodegradable. However, gelatin hydrogels lose integrity at body temperature without crosslinking. In this study, gelatin hydrogels were crosslinked using electron beam irradiation [1]. This allows simultaneous crosslinking and sterilization while avoiding use of cytotoxic chemical crosslinkers (e.g. glutaraldehyde). In order to improve hydrogels for bone tissue regeneration, mineralization with calcium phosphate (CaP) is desirable. Advantages include bioactivity (formation of a chemical bond with adjacent bone following implantation) and mechanical reinforcement. In this study, the enzyme Alkaline Phosphatase (ALP) was incorporated into electron beam-crosslinked gelatin hydrogels to induce their mineralization with CaP [2]. Subsequently, both physiochemical and biological properties were evaluated.

Materials and Methods

Hydrogels were produced by Riedel publications – ALP was added (0, 1.25 or 2.5 mg/ml) to an 8mg/ml solution of type I gelatin in ddH_2O. This solution swelled for 1 hour at room temperature, and was subsequently heated to 37°C, poured into moulds, and allowed to polymerise for 12 hours at 6°C.
Samples were irradiated, under cooling, by a linear electron accelerator.
Samples were subsequently incubated in either ddH_2O or 0.1 M CaGP for 14 days.
Mineralization was assessed by SEM, Raman, FTIR and ICP-OES
5000 MG63 osteoblast-like cells were seeded onto hydrogels, and at intervals of 1, 4 and 7 days, the morphology, proliferation and adhesion of cells on hydrogels were observed.
Mechanical testing was also performed.
All statistical significance was evaluated using one-way ANOVA on SPSS software.

Results and Discussion

SEM (FIG. 1.) illustrated the formation of CaP deposits in hydrogels containing ALP, which was further supported by FT-IR, Raman, and ICP-OES (data not shown). Mineralisation also coincided with an elevated compressive modulus (data not shown).
Cell culture (FIG. 2 and 3) demonstrated that cells were still alive, and metabolically active, at day 7 of cell culture, even at the highest ALP concentrations.
Electron irradiation of gelatin hydrogels, coupled with enzymatic mineralisation provided a successfully mineralised, stable hydrogel, which maintained biological activity. Therefore, the present method is promising for future research into bone tissue regeneration materials.

Conclusions

Gelatin hydrogels enriched with ALP and crosslinked by electron beam irradiation were successfully mineralized with CaP, leading to mechanical reinforcement. Electron beam-crosslinked hydrogels, both mineralized and unmineralized, supported MG63 cell adhesion and proliferation.

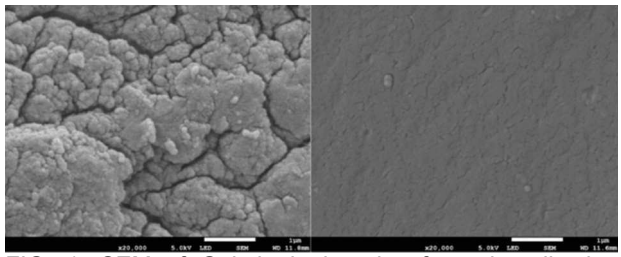

FIG. 1. SEM of Gelatin hydrogels after mineralisation (14d). Left: 2.5mg/ml ALP in CaGP. Right: 0mg/ml ALP in ddH_2O.

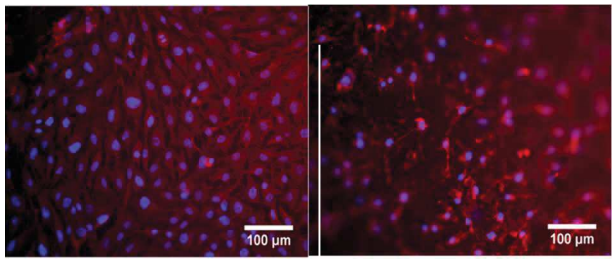

FIG. 2. Fluorescent microscopy of 0 mg/ml ALP in H_20 day 7 (left) and 2.5 mg/ml ALP in CaGP (right) stained with Texas red (red) and Hoechst (blue).

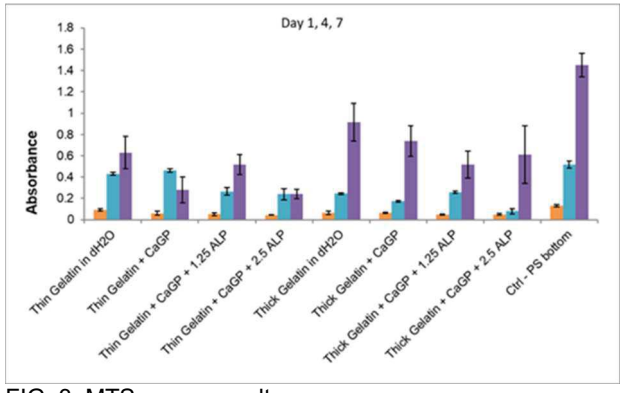

FIG. 3. MTS assay results.

Acknowledgments

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