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Article

Cell-Laden Biomimetically Mineralized Shark-Skin-Collagen-Based 3D Printed Hydrogels for the Engineering of Hard Tissues

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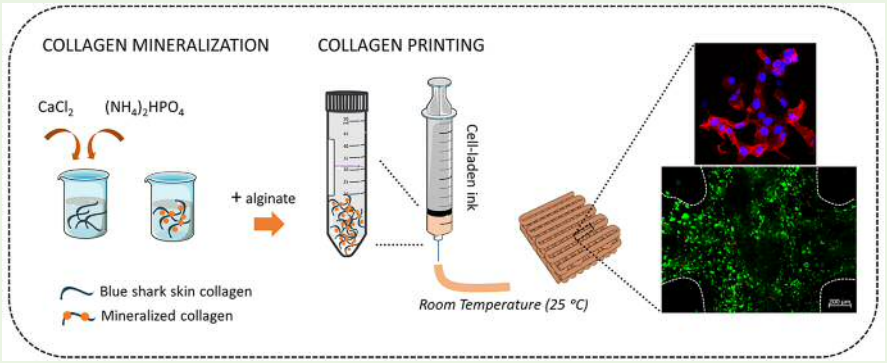

ABSTRACT: Mineralization processes based on coprecipitation methods have been applied as a promising alternative to the most commonly used methods of polymer−ceramic combination, direct mixing, and incubation in simulated body fluid (SBF) or modified SBF. In the present study, for the first time, the *in situ* mineralization (ideally hydroxyapatite formation) of blue shark (*Prionace glauca* (PG)) collagen to fabricate 3D printable cell-laden hydrogels is proposed. In the first part, several parameters for collagen mineralization were tested until optimization. The hydroxyapatite formation was confirmed by FT-IR, XRD, and TEM techniques. In the second part, stable bioinks combining the biomimetically mineralized collagen with alginate (AG) (1:1, 1:2, 1:3, and AG) solution were used for 3D printing of hydrogels. The addition of Ca^{2+} ions into the system did present a synergistic effect: by one side, the *in situ* mineralization of the collagen occurred, and at same time, they were also useful to ionically cross-link the blends with alginate, avoiding the addition of any cytotoxic chemical cross-linking agent. Mouse fibroblast cell line survival during and after printing was favored by the presence of PG collagen as exhibited by the biological performance of the hydrogels. Inspired in a concept of marine byproduct valorization, 3D bioprinting of *in situ* mineralized blue shark collagen is thus proposed as a promising approach, envisioning the engineering of mineralized tissues.

KEYWORDS: *in situ mineralization, 3D printing, bioprinting, mineralized tissues applications, marine biomaterials*

INTRODUCTION

Composite 3D structures based on collagen−apatite materials have been widely employed in the tissue engineering (TE) field, with the vision of mineralized tissues' regeneration.[1] Collagen has been receiving increasing attention as a biomaterial for use in regeneration of several tissues, since it is a key protein sustaining the extracellular matrix (ECM) structure and comprises RGD (arginine−glycine−aspartic acid) domains, which positively affect cell attachment, migration, and growth by its specific interactions with cell integrin receptors.[2,3] Apatite-based materials have been used due to their intrinsic excellent biocompatibility and bioactivity, encouraging cell adhesion, proliferation, and osteogenic differentiation.[1] Hydroxyapatite (HAp), the major inorganic component of bone tissue, is usually the first option in scaffold production for bone regeneration.[1] Considering the excellent properties of calcium phosphates for hard tissue applications, various methods of polymer−ceramic combination have been explored and applied. Direct mixing of calcium phosphate powder with polymer solution or scaffold immersion in simulated body fluid (SBF) or modified SBF are the most used techniques. However, both methods bring disadvantages. Direct mixing is an uncontrolled method and can result in a

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