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Mineralization of peptide amphiphile nanofibers and its effect on the differentiation of human mesenchymal stem cells

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ABSTRACT

One of the important targets in regenerative medicine is to design resorbable materials that can promote formation of new bone in large skeletal defects. One approach to this challenge is to use a bioactive and biodegradable organic matrix that can promote cellular adhesion and direct differentiation. We have here studied matrices composed of peptide amphiphiles (PAs) that self-assemble into nanofibers and create self-supporting gels under cell culture conditions. The bioactivity of PAs was designed by incorporating in their peptide sequences phosphoserine residues, to promote hydroxyapatite formation in the culture medium, and the cell adhesion epitope RGDS. In osteogenic medium supplemented with calcium the PA nanofibers were found to nucleate spheroidal nanoparticles of crystalline carbonated hydroxyapatite approximately 100 nm in diameter. This mineralization mode is not epitaxial relative to the long axis of the nanofibers and occurs in the presence of serine or phosphoserine residues in the peptide sequence of the amphiphiles. Mixing of the phosphoserine-containing PAs with 5 wt.% RGDS-containing PA molecules does not inhibit formation of the mineral nanoparticles. Quantitative real time reverse transcription polymerase chain reaction and immunohistochemistry analysis for alkaline phosphatase (ALP) and osteopontin expression suggest that these mineralized matrices promote osteogenic differentiation of human mesenchymal stem cells. Based on ALP expression, the presence of phosphoserine residues in PA nanofibers seems to favor osteogenic differentiation.

1. Introduction

The current gold standard for bone replacement is an autologous graft harvested from the iliac crest [1–3]. The problematical issues with autografts include defect size limits, complications with donor site morbidity, and pain. Alternatives include the use of allografts and a variety of bioceramics [4]. These alternatives also have considerable limitations, including a limited number of donors and the high cost of allografts, and the poor mechanical properties and integration of ceramics. Therefore, there is a significant need to create bioactive materials that could effectively fill bone defects and be fully resorbable as they promote bone regeneration. There has been extensive work on biomimetic materials in the context of mineralization that could be considered to develop our vision for bone replacement [5].

Our laboratory has been interested for over a decade in the molecular design of biomimetic and biodegradable matrices that can promote bone formation. We reported earlier on materials known as organoapatites [6–10] and our more recent focus has been on a class of materials known as peptide amphiphiles (PAs) [11–15]. The PAs are broadly suitable for regenerative medicine since their bioactivity can be molecularly tailored. Previous work by our laboratory [11] demonstrated the templated formation of hydroxyapatite on PA nanofibers bearing phosphorylated serine (S(P)) residues, which mimics the high occurrence of these amino acids in phosphophoryn, an important protein in the formation of dentin [16]. There has also been interest in developing mixed systems of PAs, allowing for the molecular control of epitopes presented on the exterior of the nanofiber surface. In this work we were interested in incorporating PA nanofibers containing the biological adhesion epitope Arg–Gly–Asp–Ser (RGDS) at controlled concentrations in addition to phosphorylated serine residues to template hydroxyapatite. Indeed, RGDS and other binding domains have been incorporated into peptide amphiphiles as an approach to improve cellular adhesion [17,18]. Furthermore, our objective in this work has been to probe the response of cells to mineralized

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