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Review Article

Umbilical Cord Stem Cells' Osteogenic Differentiation and Mechanical Properties as a Result of Electrospun Submicron Fibers in a Calcium Phosphate Cement Scaffold

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Abstract

In the bone defect, calcium phosphate cements (CPCs) can be injected and self-set. There are no reports of stem cell seeding on CPCs with Electrospun submicron fibers, according to a literature search. For the first time, this study wanted to find out how Electrospun fibers in CPC affected mechanical properties as well as the proliferation, osteogenic differentiation, and mineralization of human umbilical cord mesenchyme stem cells (hUCMSC). Electro spinning was used to produce poly (PLGA) fibers with an average diameter of 650 nm. Tetra calcium phosphate, dicalcium phosphate anhydrous, and chitosan lactate were the fibers that were included in CPC. There were 0%, 2.5%, 5%, and 10% fiber volume fractions. Work-of-fracture (toughness) and flexural strength of CPC with 10% fibers were twice as high as those of CPC without fibers. While attaching to the Electrospun fiber-CPC scaffolds multiplied rapidly and produced bone minerals. When the fiber volume fraction in CPC was increased from 0% to 10%, the expressions of alkaline phosphatase, osteocalcin, and collagen I in hUCMSC were doubled, and mineralization was also increased by 40%. The fiber-CPC scaffold's high surface area and biomimetic properties were blamed for the improved cell function. In conclusion, the incorporation of submicron fibers into CPC significantly enhanced its toughness and strength.

Keywords: Electrospun fibers, Calcium phosphate cement, Human cord stem cells, Osteogenic differentiation, Strength and toughness, Bone tissue engineering

INTRODUCTION

This essential requirement could be met through tissue engineering. Therapies based on stem cells are the subject of intense research (Bohner M, 2010). Mesenchyme stem cells from human bone marrow may be used to regenerate bone. However, their harvest is an invasive procedure, and as they age and develop conditions like arthritis and osteoporosis, their proliferation and differentiation decrease. Compared to human BMSCs, human umbilical cord mesenchymal stem cells (hUCMSC) are more primitive. Umbilical cords can be collected for a low price and are inexhaustible (Ginebra MP, 2006). Adipocytes, osteoblasts, chondrocytes, neurons, and endothelial cells all developed from hUCMSC. In pilot animal studies, they were not tumorigenic and exhibited a high degree of plasticity and developmental flexibility without immunorejection. For bone tissue engineering purposes, hUCMSC and calcium phosphate cement scaffolds were recently combined. It has been demonstrated that Electrospun fiber scaffolds boost mineral synthesis, cell differentiation, and proliferation. In vivo, cells are housed in an extracellular matrix of collagen fibers and Nano globules, which is mimicked by fibrous scaffolds. Consequently, it was demonstrated that scaffolds with Nano scale or submicron features significantly improved both tissue regeneration in vivo and cell function in vitro (Johnson PC, 2007). Additionally, biomimetic scaffolds were produced when calcium phosphate minerals were

incorporated into the polymer fibers. When compared to pure chitosan scaffolds, the incorporation of hydroxyapatite nanoparticles into chitosan Nano fibrous scaffolds resulted in more bone formation. Calcium phosphate (Cap) bio ceramics are another important class of scaffolds for bone repair alongside polymeric fibers (Salgado AJ, 2004). Because of their similarity to bone minerals, they are well-suited for biocompatibility. Cap implants are bioactive and can provide an ideal environment for osteoblast colonization and cellular reaction in vivo, resulting in a functional bone-implant bond. Calcium phosphate cements, which are injectable and can self-set in situ to provide intimate adaptation to complex-shaped defects, are among the most promising Cap materials. In 1986, the first calcium phosphate cement (CPC) was developed, and in 1996, the Food and Drug Administration (FDA) granted it approval to treat human craniofacial defects. However, CPC is "limited to the reconstruction of non-stress-bearing bone" because of its fragility and weakness. For the best of both worlds, it would be highly desirable to combine CPC with Electrospun fibers: CPC's mechanical stiffness, bioactivity, and paste inject ability; and the large surface area that Electrospun fibers provide for cell attachment and fiber reinforcement. The Electrospun fibers have the potential to toughen the otherwise brittle CPC for load-bearing bone repairs once they are incorporated into cement like CPC and the paste is hardened (Salinas CN, 2008). Vicryl suture fibers, which are poly fibers, were incorporated into CPC in previous studies. Single fibers with a diameter of 14 millimetres braided these suture fiber bundles, which had diameters of 322 and 198 millimetres, respectively. The submicron fibers produced by electropspining techniques were significantly smaller than these fibers. Only one paper on the incorporation of Electrospun fibers into CPC was found in a literature search. The use of poly and poly fibers in that study significantly increased CPC's porosity and work-of-fracture (Benoit DS, 2008). In the preceding study, cell seeding was not mentioned. As a result, it has not been documented how the incorporation of Electrospun submicron fibers into CPC affected the proliferation and differentiation of stem cells.

Electrospun fiber-CPC composite scaffold development

TTCP particles ranging from 1 to 80 m, with a median size of 17 m, were produced by grinding the reactant. The DCPA was ground until the median particle size of 1 m was achieved. The CPC powder was made by mixing DCPA and TTCP powders in a 1:1 molar ratio (Mendes SC, 2002). The CPC liquid was created by combining water and chitosan lactate at a chitosan mass fraction of 15%. Chitosan could be added to CPC to give the paste fast-setting and washout resistance, as well as improve CPC's mechanical properties (Stenderup K, 2003). The liquid chitosan was used to make all of the scaffolds in this study. A non-woven mat was Electrospun from submicron poly (PLGA) fibers at a ratio of 50:50 between poly (lactic acid) (PLA) and poly (glycolic acid) (PGA). The parameters were taken from previous research. In brief, a PLGA polymer solution (10% by weight) with an inherent viscosity of 0.8-1.2; PolySciences, Warrington, PA) was made by mixing dichloromethane (DCM) and dimethylformamide (DMF) in a binary ratio for 24 hours with constant stirring. A 10 mL syringe with a needle of 21 gauges was used to inject the solution into the container. An aluminium collecting plate was 12.5 centimetres away when the syringe was inserted vertically into a syringe pump. A high-voltage power supply was used to apply a voltage of 13 kV, and an electric field existed between the needle tip and the aluminium collecting plate. A non-woven mat was formed on the collection plate as a result of pumping the polymer solution from the syringe at a flow rate of 0.6 mL/h. Because a previous study demonstrated that 3-mm fibers mixed in the CPC paste could be injected through a 10-gauge needle, the fiber mat was cut into squares measuring 3 mm x 3 mm with a sharp razor blade and a ruler (Rodriguez JP, 2000). The Electrospun fibers were also cut into 3-mm pieces for use in calcium phosphate cement in another previous study. At a mass ratio of 2:1, the CPC powder and chitosan liquid were mixed together. The CPC-chitosan paste was then mixed with the fibers to create a cohesive paste. A spatula was used to mix each specimen on a flat glass slab. In accordance with previous research, the CPC paste was poured into a stainless steel mold after being randomly mixed with the fibers for about a minute. Tests were conducted on the following fiber volume fractions: 0%, 2.5%, 5%, and 10%. Due to the paste's inability to flow for injection applications, fractions of fiber volume greater than 10% were not utilized (Suzuki Y, 2001). For four hours, the specimens were incubated in a humidor at 37 °C. In order for the CPC to develop sufficient strength to withstand the forces required to push the specimen out of the mold, an incubation period of four hours was carried out, whereas the initial setting of CPC-chitosan took approximately seven minutes. Before mechanical testing, the specimens were deformed and submerged for 20 hours in a physiological-like solution.

DISCUSSION

The fiber volume fraction in CPC had an impact on mineralization, Osteogenic differentiation, and cell proliferation. Electrospun fibers were the subject of previous tissue engineering research because fibrous scaffolds resemble the extracellular matrix, which contains collagen fibers. Electro spinning polymeric fibers like micro porous, non-woven poly, and poly (PLA)-gelatine as well as electropspining collagen onto starch-based fiber meshes were the focus of numerous studies. Bio ceramic/ polymer composite fibers were developed in other studies to combine the toughness of polymers with the bioactivity and hydrophobicity of bio ceramics. The hydroxyapatite-collagen-chitosan fibers, hydroxyapatite fibers, and hydroxyapatite-chitosan fibers are two examples. In animal models, the addition of a bio ceramic component like CaP

to the polymer fibers led to increased new bone formation, enhanced mineralization, and improved cell proliferation. This is probably because adding calcium phosphate minerals to the fibers made a biomimetic matrix, since the ECM of bones also has calcium phosphate minerals and submicron fibrous features. Because the CPC paste can be easily mixed with a variety of compositions and amounts of fibers, injectable CPCs are a promising matrix for the creation of scaffolds with submicron fibrous features. The fibers provide reinforcement and a larger surface area to facilitate cell attachment, while the CPC provides bioactivity. Indeed, a recent study found that Electrospun fiber reinforcement significantly increased the work-of-fracture of calcium phosphate cement. The work-of-fracture and flexural strength of the CPC with 10% fibers were nearly 10-fold higher than those of the CPC without fibers in this study. An injectable polymeric carrier for cell delivery had a compressive strength of approximately 0.7 MPa, according to a previous study. Hydrogels for cell delivery had a tensile strength of approximately 0.07 MPa, according to other studies. It was rightly concluded that "Hydrogel scaffolds are used in no-load bearing bone tissue engineering," despite the fact that these novel materials look promising for tissue engineering.

CONCLUSIONS

Proliferation, Osteogenic expressions, and mineralization of hUCMSC were all boosted by increasing the fiber volume fraction. CPC's toughness and flexural strength both increased by a factor of two thanks to the Electrospun fibers. For cell delivery, the fiber-CPC strength was comparable to that of hydrogels and polymers by an order of magnitude. The expressions of ALP OC, and collagen I in doubled and mineralization increased by nearly when the fiber volume fraction in CPC was increased from. The Electrospun fiber-CPC scaffold's biomimetic and large surface area were cited as the underlying mechanism. As a result, it appeared that Electrospun fiber incorporation of submicron-scale fibrous features to CPC was a useful strategy for enhancing stem cell Osteogenic differentiation and mineralization.

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