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Engineering tubular bone constructs

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Abstract

Cell-sheet techniques have been proven effective in various soft tissue engineering applications. In this experiment, we investigated the feasibility of bone tissue engineering using a hybrid of mesenchymal stem cell (MSC) sheets and PLGA meshes. Porcine MSCs were cultured to a thin layer of cell sheets via osteogenic induction. Tube-like long bones were constructed by wrapping the cell sheet on to PLGA meshes resulting in constructs which could be cultured in spinner flasks, prior to implantation in nude rats. Our results showed that the sheets were composed of viable cells and dense matrix with a thickness of about 80–120 μm, mineral deposition was also observed in the sheet. *In vitro* cultures demonstrated calcified cartilage-like tissue formation and most PLGA meshes were absorbed during the 8-week culture period. *In vivo* experiments revealed that dense mineralized tissue was formed in subcutaneous sites and the 8-week plants shared similar micro-CT characteristics with native bone. The neo tissue demonstrated histological markers for both bone and cartilage, indicating that the bone formation pathway in constructs was akin to endochondral ossification, with the residues of PLGA having an effect on the neo tissue organization and formation. These results indicate that cell-sheet approaches in combination with custom-shaped scaffolds have potential in producing bone tissue.

1. Introduction

A tissue engineering approach for addressing tissue reconstruction is to engineer new tissues by using selective cell transplantation on polymer scaffolds (Langer and Vacanti, 1999). In this cell-scaffold-based tissue engineering concept, seeding of isolated cell suspensions into scaffolds often encounter problems in producing high-cell density and delivery efficiency. To achieve superior cell delivery and preservation of cell–cell contact, several researchers have attempted to use cell sheet technique for tissue engineering. Okano's group invented the use of single sheet of cultured corneal epithelial cells and multi-layered cardiomyocytes sheets for engineering transplantable corneal and myocardial tissues, respectively (Nishida et al., 2004; Shimizu et al., 2006). Cooper et al. (1993) and Pouliot et al. (2002) utilized dermal fibroblasts sheets for skin and L'Heureux et al. (1998) reported human blood vessel engineering using endothelial and smooth muscle cell sheets.

Current cell-sheet techniques provide sheets, which are strong enough to allow careful manipulation in a laboratory to produce stacked or wrapped constructs (Yang et al., 2005), however, they contract extensively upon detachment from culture surfaces and lack the mechanical strength demanded for bigger bone defects. Moreover, limited cells types are reported to form cell-sheet and most are terminally differentiated cells with inadequate lifespan and differentiation potential. The drawbacks of cell sheet techniques restrict their application in engineering large-size tissues, especially bone tissue. To overcome these problems, cell-sheet techniques can be combined with scaffolds used in current bone engineering strategies and this combination could achieve advanced cell delivery, which is not possible using the traditional cell suspension or hydro gel systems, and can result in dense, mineralized tissue formation.

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In bone tissue engineering fields, polymer scaffolds are considered promising bone substitutes for bone regeneration due to superior mechanical properties and biodegradability (Hutmacher, 2001). Synthetic polymers are easy to process into predetermined shapes. Amongst them, Poly lactic-co-glycolic acid (PLGA) has been well characterized and has shown good biocompatibility and fast degradation, and is possibly the most widely used synthetic polymer in the biomedical area (Griffith and Naughton, 2002; Hutmacher, 2001). Studies demonstrated that bone marrow mesenchymal stem cells (MSC) seeded on PLGA-derived foam or scaffolds can lead to mineralized tissue formation. However, a study repairing rabbit defects revealed less optimal neo bone growth in PLGA-derived scaffolds, which may relate to the lower efficiency of cell loading (Bidic et al., 2003).

In order to fully utilize the advantages of cell-sheet techniques and overcome cell-seeding problem in polymer scaffolds, this study exploited the feasibility of combining MSC sheets with PLGA scaffolds in bone tissue engineering. We firstly achieved the multipotent MSC sheets through stimulation of matrix formation and then wrapped the cell sheets on PLGA meshes to form tube-like constructs. The constructs underwent dynamic culture and *in vivo* implantation and results demonstrated that it was possible to regenerate bone tissue by combining MSC sheet techniques with scaffolds.

2. Materials and methods

2.1. PLGA mesh scaffold

PLGA fibers were obtained by de-braiding of Vicryl sutures (Johnson and Johnson, USA). The fibers were formed into thin non-woven meshes in the size of $2 \times 4\,cm^2$.

2.2. Cell isolation and culture

Three-month-old Duroc/Yorkshire cross pigs were obtained from the animal holding unit of National University of Singapore. The study has been reviewed and approved by the animal holding unit of National University of Singapore. Porcine bone marrow MSC were isolated and cultured as reported previously (Chen et al.). Briefly, Porcine MSCs were aspirated from marrow and after gradient centrifugation, cells were cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, Invitrogen, CA, USA) containing 2% fungizone (Sigma, St. Louis, MO, USA) and 1% antibiotics (100 μg/ml penicillium and 100 μg/ml streptomycin) at 37 °C and 5% CO_2 humidified environment. Cells of the first passages were used for all experiments. Cells were seeded and cultured in $150\,cm^2$ flasks until confluent.

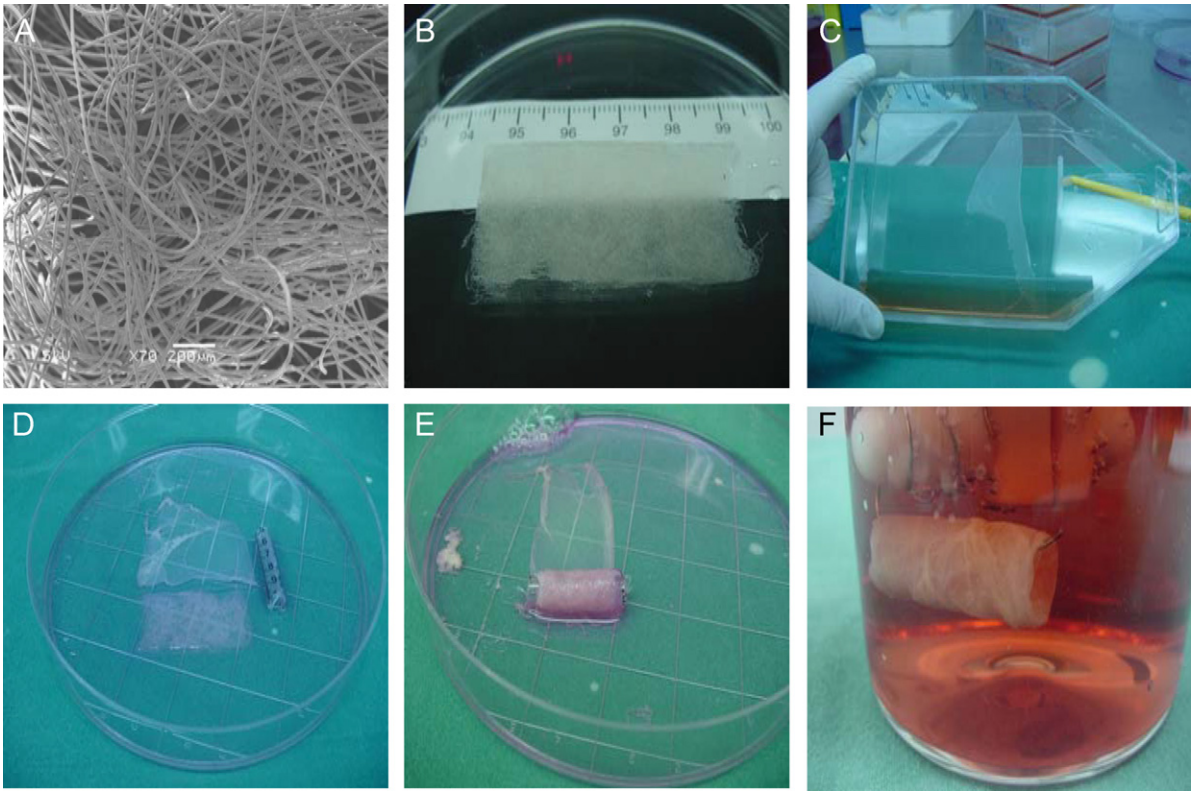

Fig. 1. PLGA fiber and mesh (A, B) and the process of PLGA-cell construction (C, D, E) with the constructs cultured in spinner flask (F). (A) SEM image of a PLGA mesh composed of fibers of 14 μm. (B) Non-woven mesh sized 4×2 cm, with a thickness of 200 μm. (C) The preparation of cell sheet derived from continuous culture of MSCs and sheet was physically detached from flask with cell scraper. (D) Cell sheet, PLGA mesh and a segment of syringe as supporter. (E) Construction of sheet mesh through wrapping of cell sheet around the PLGA mesh. (F) Cell sheet and PLGA composite in the spinner flask for dynamic culture with about 15 rounds per minutes. Scale bar: (A) 200 μm.