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The combined effects of continuous passive motion treatment and acellular PLGA implants on osteochondral regeneration in the rabbit

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ABSTRACT

We investigated the active role of clinical rehabilitation in osteochondral regeneration using continuous passive motion (CPM) treatment together with acellular PLGA implants. CPM treatment was performed and compared with immobilization (Imm) treatment and intermittent active motion (IAM) treatment upon full-thickness osteochondral defects either with or without an PLGA implant in the PI (PLGA-implanted) and ED (empty defect) models. The PI and ED tests were performed in 38 rabbits for 4 and 12 weeks. At the end of testing, the PI-CPM group had the best regeneration with nearly normal articular surfaces and no joint contracture or inflammatory reaction. In contrast, degenerated joints, abrasion cartilage surfaces and synovitis were observed in the Imm and IAM groups. The achieved bone volume/tissue volume (BV/TV) ratio, which was measured using micro-CT, was significantly higher in the CPM group compared with the Imm and IAM groups; in particular, the performance of the PI-CPM group exceeds that of the ED-CPM group. The thickness of the trabecular (subchondral) bone was visibly increased in all of the groups from 4 through 12 weeks of testing. However, a histological analysis revealed differences in cartilage regeneration. At week 4, compared with the ED samples, all of the PI groups exhibited better collagen alignment and higher GAG content in the core of their repaired tissues, particularly in the PI-CPM group. At week 12, sound osteochondral repair and hyaline cartilaginous regeneration was observed in the PI-CPM group, and this was marked by type II collagen expression, osteocyte maturation, and trabecular boney deposition. In contrast, the PI-Imm and PI-IAM groups exhibited fibrocartilaginous tissues that had modest GAG content. In summary, this study demonstrates that early CPM treatment together with acellular PLGA implantation has significant positive effects on osteochondral regeneration in rabbit knee joint models.

1. Introduction

Treating articular cartilage injuries is a complex challenge for both orthopedic surgeons and rehabilitation specialists. Although tremendous progress has been made in the field of cartilage repair, current clinical therapies, including hyaluronan injection, subchondral drilling, autologous chondrocyte implantation and mosaicplasty, still encounter obstacles such as fibrocartilage tissue formation and poor integration into the host [1–3]. To address these challenges, engineered regenerative medicine has begun to play a promising role in providing better solutions.

Generally speaking, engineered regenerative medicine comprises the following two aspects: first, the implantation of an engineered graft that provides a temporary matrix for tissue development in the repair regions; and second, the application of engineered stimuli that inspire and promote host tissue regeneration and remodeling.

In addition to the use of auto- or allo-grafts or an acellular extracellular matrix (ECM), enormous artificial grafting substitute components are currently available and include organic and inorganic-based materials. For example, in the clinic, osteochondral autograft transplantation using the mosaicplasty technique is a gold standard graft; however, these methods are plagued by limited

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donor sources, site morbidity, pain and the risk of infection as well as poor integration between the host and donor tissues. As a solution, engineered grafting substitutes have been developed and are fabricated on scaffolds that are made of synthetic materials. Due to the higher compressive load that is needed at the knee joint during normal activities, when specifically targeting articular osteochondral repair, the scaffolding material must possess superior mechanical properties as well as favorable biocompatibility and biodegradability. Poly(lactide-co-glycolide) (PLGA) is an FDA-approved synthetic biomaterial that has been applied to the regenerative medicine field due to its superiority in all the aforementioned properties. PLGA has also been used extensively in preclinical studies of ligament, tendon, cartilage, and bone regenerative medicine [4–7].

In regenerative medicine, another substantial benefit is provided by the addition of simulated physical stimuli that play critical roles in the inspiration and facilitation of self-regeneration. With regard to cell-based i*n vitro* culture strategies, static culture methods often lead to progressive cell death and inactive ECM synthesis due to insufficient nutritional uptake and waste transportation, particularly in long-term culture conditions. In contrast, alternative methods using bioreactor systems, such as spinner flasks, perfusion systems, rotating-wall vessels, pulsatile flow, and hydrostatic and hydrodynamic pressure, have essentially addressed the aforementioned limitations [8–15]. However, the use of traditional tissue-culture bioreactors is largely limited to *in vitro* processing. A continuous passive motion (CPM) strategy has been developed to provide *in situ* physical stimuli to the transplant recipients, including animal models and patients, directly at the operational site. The underlying principal of this therapeutic motion strategy is based on the physical promotion of synovial fluid that directly benefits articular cartilage with improved nutritional supply. CPM was initially introduced to animal models by Salter et al. [16], in which they adopted empty defects (ED) in rabbit models receiving CPM, immobilization (Imm) and intermittent active motion (IAM) treatments and found that the CPM groups had improved regenerative performance relative to the other groups in terms of their radiological, histological, and biochemical outcomes [16]. In clinical rehabilitation, the evidence-based medical effect of CPM has been reported to be capable of relieving pain [17,18], suppressing the inflammatory process of arthritis [17–19], and increasing functional activity [20–22] and cell proliferation in joint defect regeneration [16,23,24], particularly in early intervention.

To date, the effect of applying CPM in an animal model of cartilage repair has been investigated only in either ED models [16,23,24] or autogenous periosteal grafts [25–27]. In this study, we combined the functionalities of engineered acellular PLGA grafts and post-operative physical treatment via short-term CPM for the repair of full-thickness osteochondral defects in rabbit knee joint models.

2. Materials & methods

2.1. Fabrication and characteristics of porous PLGA scaffolds

The salt-leaching technique was used to generate a porous PLGA (lactide/glycolide ratio of 85/15, molecular weight 50–75 kDa) (Sigma, St. Louis, MO) scaffold. Porogen, consisting of sodium chloride particles with 300–500 μm in diameter, was selected through a sieve. In brief, 4 ml of 20% w/v PLGA chloroform solution was first mixed with 7.2 g sodium chloride. This mixture was poured into 3 cylinder molds and lyophilized at −20 °C for one day to remove the chloroform and form the PLGA sponges. To dissolve the porogen, the cylinder sponges were then immersed in deionized water. Finally, the PLGA sponges were cut into cylinders that were 3 mm in height and 3 mm in diameter. The morphology of the pore structure, the interconnections and the pore size of the PLGA scaffolds were observed using a scanning electron microscope (Philips, XL-40FEG, Netherland). Pore size was determined using the ImageJ software program. The porosity of the scaffolds was calculated as the difference between the bulk density and true density of a PLGA sponge cylinder.

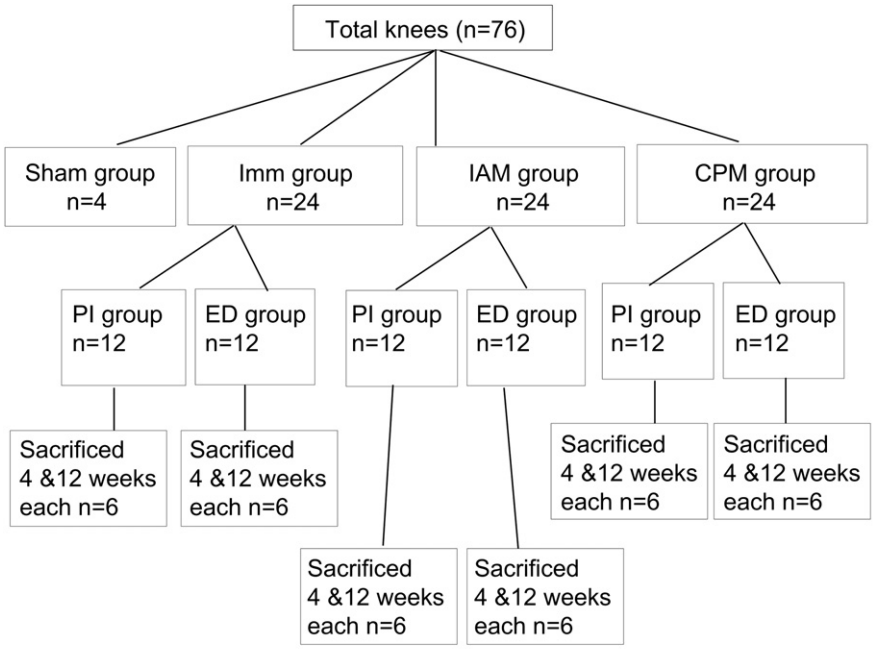

Fig. 1. A schematic diagram of the studied design and abbreviation of group names.