Biotechnology and Bioprocess Engineering 2006, 11: 550-552 ©KSBB

Chondrogenic Properties of Human Periosteum-derived Progenitor Cells (PDPCs) Embedded in a Thermoreversible Gelation Polymer (TGP)

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Abstract Periosteum-derived progenitor cells (PDPCs) were isolated using a fluorescence-activated cell sorter and their chondrogenic potential in biomaterials was investigated for the treatment of defective articular cartilage as a cell therapy. The chondrogenesis of PDPCs was conducted in a thermoreversible gelation polymer (TGP), which is a block copolymer composed of temperature-responsive polymer blocks such as poly(*N*-isopropylacrylamide) and of hydrophilic polymer blocks such as polyethylene oxide, and a defined medium that contained transforming growth factor-β3 (TGF-β3). The PDPCs exhibited chondrogenic potential when cultured in TGP. As the PDPCs-TGP is an acceptable biocompatible complex appropriate for injection into humans, this product might be readily applied to minimize invasion in a defected knee.

Keywords: chondrogenesis, periosteum-derived cells, thermoreversible gelation polymer

The use of different biomaterial carriers to create 3-dimensional cultures has in recent years included fibrin, polyglycolic and polylactic acid polymers, and alginate [1]. These materials, however, require follow up secondary surgical procedures, and are know to induce considerable pain after transplantation. Thus, we report here on our efforts to develop an ideal injectable scaffold that would relieve patient suffering along with minimal invasion of a defected site.

Recently, collagen gel [2], Matrigel [3], and a thermoreversible gelation polymer (TGP) [4-6], all of which can be directly injected into patients, have been employed to maintain chondrocyte phenotypes in three-dimensional cultures. Since collagen gel and Matrigel are prepared from bovine or mouse tumors, respectively, it is not possible to completely eliminate a chance of infection with bovine spongiform encephalopathy. In contrast, TGP is controlled chemically synthesized material, and as such is completely free of pathogens such as prions [4]. Furthermore, TGP is a temperature-sensitive polymer solution, which forms a hydrogel via the association of copolymer chains when exposed to *in vitro* or *in vivo* environments at 37°C [5]. TGP is a block copolymer composed of temperature-responsive polymer (TRP) blocks such as poly(*N*-isopropylacrylamide) or polypropylene oxide and of hydrophilic polymer blocks such as polyethylene oxide. The TGP remains as a liquid at low temperature but reverts to a gel immediately upon heating and returning to a liquid state when cooled. Such a dynamic transition occurs because the TRP and hydrophilic polymer blocks are both water-soluble. When the liquid solution is heated above the sol-gel transition temperature, TRP blocks are converted into a water-insoluble hydrophobic state and associate between separate molecules due to hydrophobic interaction [6]. Due to the favorable biocompatibility properties of TGP, it has been speculated to be useful as an acceptable biomaterial for cell-based therapies [7].

Autologous chondrocyte transplantation (ACT) procedures are usually limited by the availability of autologous cells, and as a result the development of a new cell source is necessary. The following criteria should be considered when considering such alternative cell sources: autologous source of origin, chondrogenic potential, relative ease of access, cell expandability, cell phenotypic stability of the cells throughout the expansion procedures, and minimal possible surgical morbidity [8]. We selected periosteum to be used as an alternative cell source on the basis of these criteria for the conduct of this study. Periosteum is characterized by a substantial quantity of multipotent progenitor cells capable of selectively differentiating into osteogenic or chondrogenic lineages, depending on their *in vitro* culture conditions regardless of their passage number or the donor age [8,9].

In this study, periosteum was prepared from men and women undergoing surgical knee replacement procedures and harvested from the proximal tibia tissue (donors age range 40 to 72). The periosteum was briefly rinsed twice with phosphate buffered saline (PBS) supplemented with an antibiotic-antimycotic solution (Sigma, St. Louis, MO,

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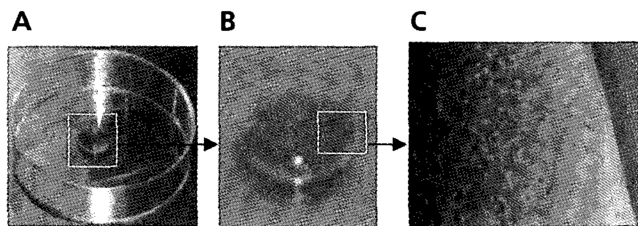

Fig. 1. The features of the PDPCs embedded in the TGP (A), (B) and observation of the PDPCs in the TGP by microscopy (C).

USA) and then dissected into small slices. The periosteum-derived progenitor cells (PDPCs) were then isolated according to the previously described procedure [10].

The isolated PDPCs were then mixed thoroughly in TGP (Mebiol® gel, Technomart Inc., Seoul, Korea) to yield a final cell density of $1 \times 10^7 \sim 2 \times 10^7$ cells/mL. To induce the desired chondrogenic differentiation, the PDPCs were then cultivated in a basal medium (hMSC Differentiation BulletKit®, Cambrex Bio Science Walkersville, Inc., MD, USA) with supplements (hMSC Chondrogenic BulletKit®, Cambrex Bio Science Walkersville, Inc.) plus 10 ng/mL of TGF-β3 (R & D System, Minneapolis, MN, USA). The isolated cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% air and the media changed every three days.

Total RNA was extracted with TRIzol reagent (Invitrogen, CA, USA) and the cells lysed by the addition of 1 mL of TRIzol reagent [11]. The total RNA was isolated according to the manufacturer's instructions. Complementary DNA was synthesized from 1 μg of total RNA per each sample, using AMV reverse transcriptase. The reaction was then conducted in a final 20 μL volume that included 5 mM $MgCl_2$, 1 mM of each deoxynucleotide, 1.6 g of oligo-(dT), 50 U/mL of RNase inhibitor, and 20 U/mL of AMV reverse transcriptase in 50 mM KCl and 10 mM Tris-HCl, at a final pH of 8.3. This mixture was incubated for 10 min at 25°C, at 42°C for 60 min, heated to 99°C for 5 min, and finally flash-cooled to 4°C. PCR amplifications for the type II collagens (Col. II), Sox9, aggrecan, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (as a control) were conducted for 30 cycles. Each cycle consisted of 30 sec/95°C denaturation, 30 sec/58°C annealing, and 2 min/72°C extension, using a recombinant Taq DNA polymerase (Takara Korea Biomedical, Inc., Seoul, Korea).

The test samples were also evaluated with regarding the presence of proteoglycan by using a Blyscan glycosaminoglycan (GAG) assay kit (Biocolor, Newtownabbey, Northern Ireland). This assay is predicated on the specific binding of the cationic dye, 1,9-dimethylmethylene blue, to the sulfated GAG chains of the proteoglycans. The procedure was conducted according to the manufacturer's instructions. The samples (500 μL) were mixed with 500 μL of the Blyscan dye reagent and mixed for 30 min at room temperature. The GAG-dye complex was recovered by centrifugation, and the pellets were washed and resuspended in 1 mL of dissociation buffer. Absorbance was measured at a wavelength of 656 nm.

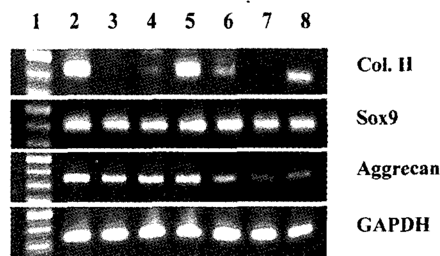

Fig. 2. RT-PCR for the chondrogenesis of PDPCs. Lane 1, 1 kb DNA ladder; lane 2, chondrocytes (P0); lane 3, dermal fibroblast; lane 4, PDPCs (P0); lanes 5~8, cells in TGP: lane 5, chondrocytes; lane 6, PDPCs cultured in basal medium with 10% FBS for two weeks; lanes 7 and 8, PDPCs cultured in chondrogenic induction medium for 1 and 2 weeks, respectively.

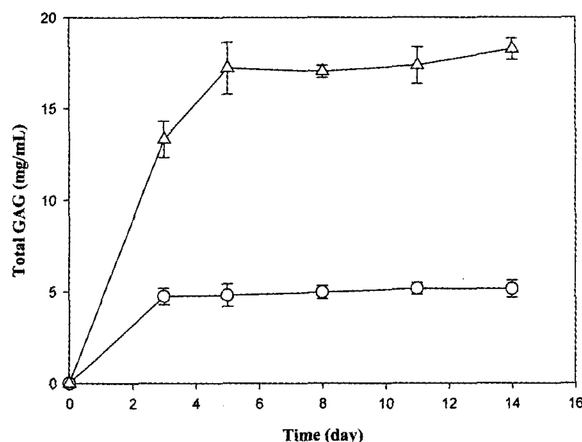

Fig. 3. Time-course profiles of the synthesis of total GAG by the PDPCs in the TGP; 10% FBS containing medium (○), chondrogenesis induction medium (△).

Fig. 1 indicates that the PDPCs were successfully embedded in the TGP, and that their rounded-cell morphology of the PDPCs was maintained. Thus, in practice, the PDPCs would be initially held within the TGP, which is transformed into the hydrogel state when exposed to an *in vitro* environment of 37°C.

The changes that occurred in the mRNA expression levels for Col. II, Sox9, aggrecan, and GAPDH of the PDPCs within the TGP are shown in Fig 2. The mRNA levels for the Col. II of the PDPCs in TGP increased gradually over two weeks of their chondrogenic induction. In marked contrast, the aggrecan levels in the TGP were observed to decrease.

The total GAG, which was a chondrocyte-specific extracellular matrix secreted by the differentiated PDPCs in the chondrogenic medium during chondrogenesis is shown in Fig. 3. The amount of GAG increased 3.5-fold above that measured under normal conditions. This finding also implies that the PDPCs embedded in the TGP could be differentiated into chondrocytes within a specific artificial chondrogenic environment.

In summary, we focused our efforts on the characterization of the chondrogenic differentiation of isolated PDPCs embedded within the TGP. The RT-PCR was employed to confirm the expression of chondrocyte-specific mRNA, and GAG analysis was also performed. The results of the present study suggest that the PDPCs and TGP may have a potential clinical use as a potential new cell source, and also as an injectable scaffold for cartilage regeneration.

Acknowledgements This work was supported by the Inha Vision development program, Center for Advanced Bioseparation Technology, and by Korea Science and Engineering Foundation (KOSEF R01-2005-000-10927-0).

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[Received May 24, 2006; accepted December 18, 2006]