Journal of Bioscience and Bioengineering
VOL. 120 No. 5, 570–576, 2015

ELSEVIER

www.elsevier.com/locate/jbiosc

Comparison of manual and automated cultures of bone marrow stromal cells for bone tissue engineering

Hirokazu Akiyama,[1] Asako Kobayashi,[2] Masaki Ichimura,[1] Hiroshi Tone,[3] Masaru Nakatani,[1] Minoru Inoue,[2] Arinobu Tojo,[2] and Hideaki Kagami[2,4,*]

Medical Device Development Laboratories, Kaneka Corporation, 1-8 Miyamae-cho, Takasago-cho, Takasago, Hyogo 676-8688, Japan,[1] Tissue Engineering Research Group, Division of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan,[2] Technology Management Department, Medical Devices Division, Kaneka Corporation, 2-3-18 Nakanoshima, Kita-ku, Osaka 530-8288, Japan,[3] and Department of Oral and Maxillofacial Surgery, Matsumoto Dental University School of Dentistry, 1780 Hirookagobara, Shiojiri, Nagano 399-0781, Japan[4]

Received 27 December 2014; accepted 16 March 2015
Available online 20 April 2015

The development of an automated cell culture system would allow stable and economical cell processing for wider clinical applications in the field of regenerative medicine. However, it is crucial to determine whether the cells obtained by automated culture are comparable to those generated by manual culture. In the present study, we focused on the primary culture process of bone marrow stromal cells (BMSCs) for bone tissue engineering and investigated the feasibility of its automation using a commercially available automated cell culture system in a clinical setting. A comparison of the harvested BMSCs from manual and automated cultures using clinically acceptable protocols showed no differences in cell yields, viabilities, surface marker expression profiles, and *in vivo* osteogenic abilities. Cells cultured with this system also did not show malignant transformation and the automated process was revealed to be safe in terms of microbial contamination. Taken together, the automated procedure described in this report provides an approach to clinical bone tissue engineering.

[**Key words:** Automated cell culture system; Bone marrow stromal cells; Osteogenic potential; Bone tissue engineering; Regenerative medicine]

Substantial efforts in tissue engineering have provided innovative methods to repair or regenerate damaged tissues and organs. Yet, although many clinical trials in this field have shown promising results (1–6), several problems remain that need to be addressed before these techniques find wider clinical application in regenerative medicine. One major problem is that current clinical cell processing methods, in addition to their high cost, are dependent on manual operations, which are vulnerable to human error and microbial contamination. To achieve stable and economical cell processing, automated systems have gained interest (7).

To date, several automated cell culture systems have become commercially available (8–11), such as Auto Culture (Kawasaki Heavy Industries) (8) and CompacT SelecT (The Automation Partnership) (9–11), in which cells are cultured using open culture vessels manipulated by robotic arms incorporated into the aseptically controlled chamber. Because the robotic arm can replicate manual operations, this type of system has broad applicability for various types of cells. By contrast, the automated systems P 4C S (Kaneka, Osaka, Japan) and Quantum (Terumo BCT) (12) employ closed culture vessels, which confer enormous advantages in terms of compactness and the relatively low cost of machinery. The P 4C S was developed based on the prototype system reported by Kato et al. (13) and performs cell culture using an original disposable tubing set containing a single culture flask. One of the characteristic features of this culture system is the repeated use of a single culture flask even after cell passage, which is advantageous both for the compactness of the machinery and for stable continuous culture. Although the feasibility of this system for primary cultures of BMSCs and fibroblasts was reported, the detailed characters of cells cultured with this system have not been investigated.

Recently, bone tissue engineering has attracted significant attention because it is less invasive than autologous bone grafting and may be more effective than artificial bone substitutes (14). Preliminary results from clinical studies demonstrated the usefulness of this approach for severe atrophy of alveolar bone (15). However, for the wide acceptance and commercialization of bone tissue engineering, an automated culture system remains desirable.

In this study, we examined the feasibility of replacing the manual culture of BMSCs in the clinical setting of alveolar bone tissue engineering with an automated procedure using the P 4C S. Emphasis was placed on comparing the manually cultured cells with cells from the automated culture system. Importantly, we also examined the safety concerns regarding the microbiological contamination of automated culture and the oncogenic transformation of the cultured cells.

* Corresponding author at: Tissue Engineering Research Group, Division of Molecular Therapy, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan. Tel.: +81 3 5449 5120; fax: +81 3 5449 5121.

E-mail address: kagami@ims.u-tokyo.ac.jp (H. Kagami).

1389-1723/$ – see front matter
http://dx.doi.org/10.1016/j.jbiosc.2015.03.011

MATERIALS AND METHODS

Automated cell culture system The P 4C S (Kaneka) was developed based on the prototype system reported by Kato et al. (13). The photographs of the P 4C S are shown in Fig. 1A. The system was designed to perform cell culture in an enclosed system using a specially designed disposable tubing set containing a culture flask (culture area, 490 cm^2), air filters, and solution bags. The dimensions of the machinery are 110 cm (W) × 62 cm (D) × 63 cm (H). The upper side of the machinery consists of the cooler and the incubation units. The cooler unit maintains the media and cell detachment solution at 5°C; the incubation unit maintains the culture environment at 37°C in an atmosphere of 5% CO_2. On the lower side of the machinery, peristaltic pumps and valves are arranged to control the flow of solutions and air. The system is operated by an external personal computer.

The detailed view of the whole culture system is shown in Fig. 1B. The solution bags in the tubing set consist of a sample material bag for cell loading, medium bags for storing the culture media, a cell-detachment solution bag for storing protease (e.g., trypsin), a saline solution bag for washing cells, waste bags for storing waste liquid, and a cell collection bag for collecting the cell suspension after cultivation. The system performs the typical cell manipulations of cell loading, medium exchange, and cell harvest. For cell loading, sample materials in the sample material bag are poured into the culture flask after which medium is supplied from the medium bags. For medium exchange, the spent medium in the culture flask is discarded by pumping it into the waste bag and fresh medium is then supplied. For cell harvest, the spent medium is discarded and the cells are repeatedly washed in saline solution. Protease is supplied from the cell-detachment solution bag and the cells are incubated for a specified period of time. Thereafter, fresh medium is added to stop the protease activity and the detached cells are pumped into the cell collection bag. During culture, fresh air (5% CO_2) is periodically supplied to the culture flask through air filters in the incubation unit. In addition, images of multiple fixed positions within the culture flask are captured daily by a complementary metal-oxide-semiconductor camera arranged underneath the culture flask in the incubation unit. These manipulations are automatically performed according to the computer program. The detailed methods of these manipulations are described by Kato et al. (13).

Media Fill test Potential microbial contamination in the automated cell culture process was estimated using a Media Fill test (16,17). The disposable tubing set, consisting of a sample material bag, medium bags, and a cell-detachment solution bag, all of which were filled with trypticase soy broth (Sysmex, Kobe, Japan) as microbial growth medium, was prepared using a clean bench with a grade A environment (class 100) in a clean room. The tubing set was then transferred to the machine, located in a conventional laboratory without any air filtration system. The culture period was set to 21 days, considered to represent the worst-case scenario (longest period of machine culture). After the simulation of the 21-day machine culture and following the 14-day manual culture (35-day culture in total), the medium was sampled. Thereafter, the harvested sample was incubated for 7 days at 22–25°C followed by an additional incubation for 7 days at 30–35°C. Subsequently, the harvested medium sample was qualitatively examined for evidence of microbial growth or turbidity during incubation and at the end of the 14-day incubation. The experiment was repeated three times.

Manual and automated cultures Fresh human bone marrows, obtained from three healthy donors (25- and 28-year-old males and a 38-year-old female), were purchased from AllCells (Emeryville, CA, USA). Each bone marrow was divided into two 20-mL groups, and the manual and automated primary cultures of BMSCs were performed in parallel using the clinically acceptable protocols described below.

For manual culture, 20 mL of bone marrow aspirate was diluted four-fold with serum-free alpha-minimum essential medium (α-MEM; Life Technologies, Carlsbad, CA, USA) supplemented with 50 μg gentamicin (Fuji Pharma, Tokyo, Japan)/mL and 2.5 μg amphotericin B (Fungizone; Bristol-Myers Squibb, New York, NY, USA)/mL. Then, diluted bone marrow was plated into four T-150 flasks (Corning, New York, NY, USA) so that each flask contained 5 mL of bone marrow. After 4 days of cultivation in a 37°C, 5% CO_2 incubator, the culture medium was replaced with α-MEM supplemented with 10% fetal bovine serum (FBS; Thermo Scientific, Waltham, MA, USA) and 1 ng of basic fibroblast growth factor (bFGF; Kaken Pharmaceutical, Tokyo, Japan)/mL. Thereafter, the cells were fed twice a week with α-MEM supplemented with FBS and bFGF until they reached sub-confluence, at which time they were harvested using TrypLE Select (Life Technologies).

For automated culture using the P 4C S, 20 mL of bone marrow aspirate was poured into the sample material bag. Cell loading was then carried out to transfer

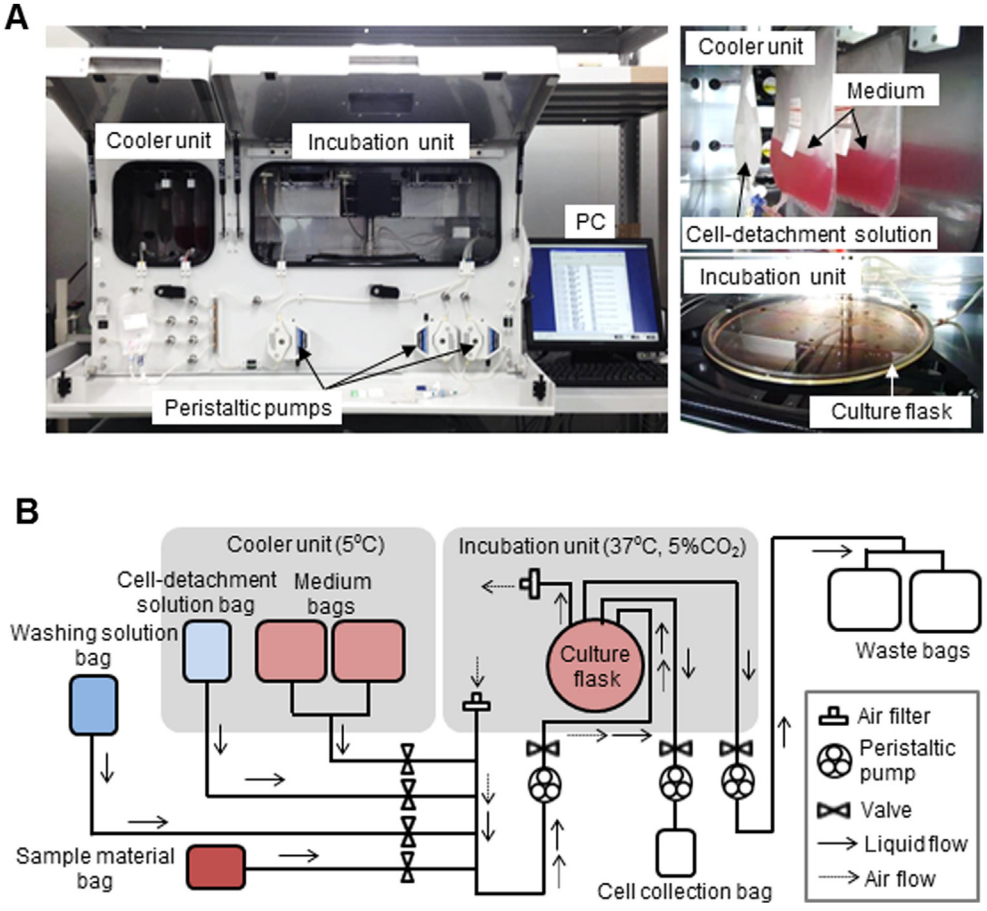

FIG. 1. Automated cell culture system. (A) Photographs of the P 4C S. The P 4C S was designed to culture cells in a closed system by using a single-use disposable tubing set containing a cell culture flask, air filters, and solution bags. (B) A detailed view of the whole culture system.