Cartilage Regeneration from Bone Marrow Cells Using RWV Bioreactor and Its Automation System for Clinical Application

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1. Introduction

Articular cartilage covers the end of bones in joints and determines the load-bearing characteristics and mobility of joints. It has a thin, smooth, low friction surface with a remarkable resiliency to compressive forces. In general, chondrocytes occupy lacunae in the matrix, and produce cartilaginous ECM (extracellular matrix), which consists of type II collagen (13%), proteoglycans (7%), and water (80%).

Cartilage defects result from aging, joint injury, and developmental disorders, causing joint pain and loss of mobility. Articular cartilage is metabolically active, however, the chondrocytes have a slow turnover rate. Thus, articular cartilage might suffer progressive damage and degeneration with a limited spontaneous repair capability. Total joint arthroplasty is the final choice of treatment, however, it is not suitable for young patients because of the limited life span of the artificial joint. Marrow-stimulating techniques such as microfracturing, multiple drilling, mosaicplasty and autologous chondrocyte implantation are clinically available for young patients, but have some limitations (Ikada, 2006). Marrow-stimulating techniques result in a fibrocartilage with less mechanical strength than hyaline cartilage and only limited repair capacity. The major problems with mosaicplasty are a limited availability of autologous tissue and donor site morbidity, the destruction of healthy non-weight-bearing tissue to repair diseased tissue. Autologous chondrocyte transplantation with a periosteal graft has shown encouraging results, however, predictability and reliability are still questionable.

Ochi et al. (2002) showed a clinical advantage of transplanting autologous chondrocytes cultured in collagen gel for the treatment of full-thickness defects of cartilage in 28 knees over a minimum period of 25 months. Arthroscopic assessment indicated that 26 knees (93%) had a good or excellent outcome. Wakitani et al. (2002) applied cell transplantation to repair human articular cartilage defects in osteoarthritis knee joints. The study group comprised 24 patients with knee OA. Adherent cells expanded from bone marrow aspirates were embedded in collagen gel and transplanted into the articular cartilage defects of 12
knees, with the other 12 knees serving as cell-free controls. Arthroscopic and histological grading scores were better in the cell-transplanted group than cell-free group. In spite of these successful clinical results to expand the clinical treatment of cartilage diseases, we need to establish a three dimensional culture technique for regenerating large cartilage tissue in vitro. One solution is to use an RWV (rotating wall vessel) bioreactor.

2. Regeneration of cartilaginous tissue from rabbit bone marrow cells under three dimensional culture by RWV bioreactor

2.1 RWV (rotating wall vessel) bioreactor

Recently, three-dimensional cell culture techniques have attracted much attention among not only cell and developmental biologists but also clinicians who have an interest in tissue engineering (Abbott, 2003). The limitations of two-dimensional culture using conventional flasks or dishes are becoming clear. In the field of tissue engineering, the chondrocyte cell is a typical example of the major difference between a flat layer of cells and a complex, three-dimensional tissue (Holtzer, 1960; Passaretti et al., 2001). Matured chondrocytes in the two-dimensional condition dedifferentiated without maintaining their phenotype and lost their original phenotype after four rounds of subculture. Clinically, a method of regenerating cartilage tissue needs to be established to treat diseases such as osteoarthritis. Thus, the development of a cell culture system for the growth of three-dimensional cartilage is important. However, problems such as necrosis due to high-density cell culture and shear stress have not yet been solved using conventional stirred fermentors. We examined the use of a rotating wall vessel (RWV) bioreactor that simulates a microgravity environment with low shear stress for cartilage tissue regeneration (Fig.1). This bioreactor generates stress by the horizontal rotation of a cylindrical vessel equipped with a gas exchange membrane. The RWV bioreactor compensates for the effect of gravity, resulting in homogenous cell growth and differentiation without sinking, and cells aggregate and form a three-dimensional tissue. The advantage of using an RWV bioreactor for tissue formation was first reviewed by Unsworth and Lelkes (1998), who discussed the benefits of growing tissues in microgravity and simulated microgravity. The formation of tissue by, for example, endothelial cells (Sanfold et al., 2002), colon carcinoma cell lines (Goodwin et al., 1992), ovarian cancer cells (Goodwin et al., 1997), osteoblasts (Qiu et al., 1999), and erythroid cells (Sytkowski and Davis, 2001), has been reported. In particular, the RWV bioreactor has been shown to stimulate chondrogenesis (Baker and Goodwin, 1997; Duke et al., 1993). Moreover, a comparison of chondrocyte cells cultured in rotating bioreactors in space (Mir space station) and on earth was reported by Freed et al. (1997a). They performed rotating cultures of bovine chondrocytes in polyglycolic acid (PGA) scaffolds and concluded that the culture on earth produced cartilage tissues closer to the natural form than that in space. In this case, the culture period for obtaining tissue was 7 months. Very recently, the chondrogenesis of human cartilage by an RWV bioreactor has been reported (Marlovits et al., 2003). Good quality cartilage tissue was formed by rotating culture from aged human articular cartilage after 90 days of cultivation. In spite of numerous studies on the formation of cartilage tissue from chondrocytes, our report (Ohyabu et al., 2006) was the first on the production of cartilaginous tissue from bone marrow-derived cells by rotating culture. A three-dimensional cell culture technique was established for the construction of large and homogenous cartilage tissues without a scaffold using bone marrow-derived cells and an RWV bioreactor.
2.2 Regeneration of cartilage tissue in vitro using rabbit bone marrow cells and an RWV bioreactor

Bone marrow cells were collected from the femora of six 10-day-old Japanese white rabbits and cultured in a standard medium consisting of Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal bovine serum for 3 weeks. The cells were resuspended in a chondrogenic differentiation medium comprising DMEM containing 10% FBS and 10 ng/mL of TGF-β (Johnstone et al., 1998) and seeded in the discoidal vessels of an RWV reactor in a CO₂ incubator. A rotatory culture was performed for 4 weeks. The rotation speed was adjusted manually in order to keep cell aggregates freely suspended within the vessel. As a control, a tube culture was performed, a kind of three dimensional culture technique developed by Holtzer and Manning (Holtzer, 1960; Manning and Bonner, 1967) and established by Johnstone et al. (1998). After the rotating culture in the RWV vessel and
static culture in the conical tube, the aggregates were harvested and prepared for histochemical and biochemical analysis.

![In vitro cartilage tissue regeneration from rabbit bone marrow cells using RWV bioreactor](image)

Fig. 3. In vitro cartilage tissue regeneration from rabbit bone marrow cells using RWV bioreactor

The rotation speed of the RWV was adjusted manually to prevent the cell aggregates from sinking in the RWV vessel. The speed was varied between 11 and 25 rpm and increased steadily for 28 days (Fig. 2). The change in speed originated from the steady increase in the mass of tissue formed in the vessel. Figure 3 shows images of the tissue formed in the RWV vessel and in the 15-mL conical tube after 1 and 4 weeks of culture. The tissues are of a cylindrical shape. The size (height/diameter) of the tissue in the RWV vessel was 1.00/0.48 cm at 1 week; 1.28/0.53 cm at 2 weeks; and 1.25/0.60 cm at 4 weeks. This kind of single tissue formed in the reactor reproducibly in the same experimental conditions. By contrast, the tissue formed in the 15-mL conical tube was smaller: 0.20/0.90 cm at 1 week; 0.20/0.55 cm at 2 weeks; and 0.28/0.74 cm at 4 weeks. The qualities of the tissues as cartilage were evaluated by histochemical methods including immunostaining of collagen type I and collagen type II and safranin-O and toluidine blue staining (Fig. 4). The staining of collagen type II was more intense in the RWV than tube culture. The results of safranin-O and toluidine blue staining are clearer as shown in Figure 4c and d. The time course of safranin-O and toluidine blue staining in the tissues formed in the tubes shows that the tissue gradually became chondrogenic. However, this change occurred faster in the RWV tissues. Even at 1 week, chondrogenesis occurred in the RWV tissues, but no sign of chondrogenesis was detected in the tissues in the tubes. At 2 weeks, the difference between the two was clearer, and at 4 weeks, chondrogenesis of the RWV tissues was confirmed by the strong and homogeneous matachromasy of safranin-O staining. Comparing the results of safranin-O staining, the RWV tissue at 1 week appeared to be in a similar stage of differentiation as the tube tissue at 4 weeks. In conclusion, we succeeded in the rapid regeneration of three-dimensional large and homogeneous cartilaginous tissue from rabbit bone marrow cells without a scaffold using a RWV bioreactor. Bone marrow cells cultured for 3 weeks were resuspended and cultured for 4 weeks in the chondrogenic medium within the vessel. Large cylindrical cartilaginous tissue 1.25 cm in height and 0.60 cm in diameter formed. Their cartilaginous properties were demonstrated by immunohistochemistry of collagen types I and II, mRNA expression of aggrecan, collagen types I and II, GAG/DNA ratio, toluidine blue, and safranin-O staining, and polarization.
Fig. 4. Immunostaining of collagen type I (a) and collagen type II (b), safranin-O staining (c), and toluidine blue staining (d) of the tissues formed in the RWV vessels and in the conical tubes at 1, 2 and 4 weeks. Bar.1 mm. (Ohyabu et al., 2006)

Despite numerous studies on the formation of cartilage tissue from chondrocytes (Baker and Goodwin, 1997; Duke et al., 1993; Freed and Vunjak-Novakovic, 1997b; Freed et al., 1997a; Marlovits et al., 2003), no report has been published on the production of cartilage tissue from bone marrow-derived cells in rotating cultures. Stem cell-derived tissue formation is a basic concept in tissue engineering. However, three-dimensional cartilage tissue has not yet to be produced in rotating cultures. Our study showed that (1) in vitro chondrogenesis was observed in 3D culture of bone marrow stromal cells and that (2) RWV culture yielded cartilaginous tissues that was superior to static culture. As shown in Figure 4, the cartilaginous tissue is homogeneous and showed no necrotic cells of large size 1.25/0.6 cm. These results are encouraging and promising for cartilage tissue engineering, and an experiment in which the tissue formed by an RWV bioreactor as transplanted to large osteochondral defects described in the next paragraph. It is true that it is the first study to
utilize bone marrow stromal cells, but we can gain a lot of benefit by using bone marrow cells. First, the number of autologous cultured chondrocytes is limited and applicable to only a limited area of cartilage damage, while a greater number of chondrocytes can be cultured from bone marrow cells (Wakitani et al., 2004). From this point of view, the clinical applications of RWV using bone marrow cells are wider than those using autologous chondrocytes. Second, bone marrow cells might be suitable for cartilaginous tissue formation by RWV culture. It has been reported that the exposure to RWV at an early stage of chondrogenesis severely limits the ability for cartilage growth, however, at a late stage of chondrogenesis, the RWV environment is beneficial and enhances growth and development using embryonic mouse pre-bone tissues (Klement et al., 2004). These results seem to contradict our results obtained using bone marrow stromal cells. Bone marrow stromal cells might be more suitable for RWV culture than embryonic cells. There are two major differences between the RWV culture and tube culture. One is the way in which the cells aggregate gathered manually in the RWV, whereas they gathered due to centrifugal force in the tube. The other point is that the unique mechanical stress due to the medium flow and gravity continued to apply to the tissue in the RWV (Klaus, 2001; Klement et al., 2004). We speculate that the major reason that superior tissue formed in the RWV was that the appropriate mechanical force was applied to the naturally gathered cells. To study the methodology of cartilage regeneration from bone marrow cells, a comparison of the tissue engineered in the RWV bioreactor with and without a scaffold would be useful. This kind of study was published by our group (Ohyabu et al. 2009).

2.3 Regeneration of cartilage tissue in vivo using rabbit bone marrow cells and an RWV bioreactor

As described in the last section, the application of a rotating wall vessel (RWV) bioreactor that simulates a micro-gravity environment with low shear stress for regenerating cartilage tissue and successfully established a three-dimensional (3-D) cell culture technique for the formation of large and homogenous cartilaginous aggregates from bone marrow-derived cells without using a scaffold. This bioreactor generates stress through the horizontal rotation of a cylindrical vessel equipped with a gas exchange membrane. The bioreactor compensates for the effect of gravity, resulting in homogenous growth and differentiation without sinking, and the cells aggregate and form 3-D tissue. This section describes the usefulness of transplanting cartilaginous aggregates formed from rabbit bone marrow-derived cells using an RWV bioreactor (Yoshioka et al., 2007).

Cylindrical defects 5x5 mm in area and 4 mm in depth were created on the patellar groove of the rabbit femur with a hand drill. For the control, the defects were left empty (Group-C: control group, n=18), whereas 3-D cartilage aggregates of 10 mm x 5 mm (height x diameter) were placed into the defects without any flap (Group-T: transplanted group, n=18) as shown in Fig.5. The rabbits were then caged and allowed to move freely without any splinting before being sacrificed 4 (n=6), 8 (n=6) and 12 (n=6) weeks after the operation. Fig.5 shows a macroscopic view of defects of the patella groove of the knee and histological view of a section of osteochondral tissue 4 weeks after transplantation. Large osteochondral defects in the knee joints were successfully repaired with hyaline-like cartilage after the transplantation of allogeneic cartilaginous aggregates formed from bone marrow-derived cells using the RWV bioreactor. As early as 4 weeks after the operation, the defects were filled with reparative tissue that resembled hyaline cartilage. The reparative tissue had a
smooth surface and there were no fibrous tissues between the reparative tissue and adjacent normal cartilage. At 8 weeks, enchondral bone had formed in the deeper portion of the reparative tissue. At 12 weeks, in some cases the intensity of staining with safranin-O was slightly reduced in comparison to that in the adjacent normal cartilage, but the reparative tissue retained its thickness. This is the first report of the rapid regeneration of critical osteochondral defects with allogeneic cartilaginous aggregates formed from bone marrow-derived cells without any scaffold using the RWV bioreactor.

Fig. 5. Transplantation of RWV tissue into osteochondral defects in rabbit knees

There are two important advantages. First, the cells are derived from bone marrow and mesenchymal stem cells (MSCs). Therefore it is possible for the cells to proliferate in a monolayer culture and then to differentiate into cartilage and bone (Pittenger et al.1999, Caplan 1991). An additional advantage to using bone marrow-derived cells in a clinical context is that they can be collected through aspiration of both sides of the iliac crest under partial anesthesia, a procedure that is very easy to perform and minimally invasive. Second, we used the RWV bioreactor as the 3-D culture system which stimulates chondrogenesis in a simulated micro-gravity environment (Unsworth et al. 1998). In previous reports (Wakitani et al. 1994, Im et al. 2001), engineered cartilage from MSCs required a scaffold for the cells to remain in the defect and to act as a support for inducing the formation of hyaline cartilage. However, using our technique, it is possible to form large and homogenous cartilaginous aggregates without any scaffold which can thus be transplanted into large osteochondral defects, which do not spontaneously regenerate in the rabbit (Shapiro et al. 1993). These aggregates have already produced an abundance of extracellular matrix and type-II collagen which was used to identify the chondrogenic phenotype in vitro. This suggests that the aggregates formed in the RWV bioreactor have the characteristics of hyaline cartilage. Rich extracellular matrix embedded chondrocytes to maintain their phenotype protecting them from dedifferentiation. In addition, we had a specific reason to choose aggregates at as early as 1 week after culture in the RWV bioreactor for transplantation. According to our in vitro
study (Ohyabu et al. 2006), the cells of cartilaginous aggregates after 1 week of culture are not mature, but consist of undifferentiated or chondrogenic precursor cells, which might exhibit plasticity to differentiate into another phenotype such as osteoblasts etc. The aggregates of such cells were influenced by various biological factors from the host bone marrow side (Engstrand 2003, Wozney et al. 1990) and interacted with adjacent cartilage (Tognana et al. 2005, Zhang et al. 2005). A suitable distribution of mechanical stress and synovial factors (Serink et al., 1997, Yanai et al. 2005) also influence the lineage of these cells. These characteristics may thus make it possible to rapidly and suitably regenerate cartilage-bone structure in vivo.

3. Rotating three-dimensional dynamic culture of adult human bone marrow-derived cells for tissue engineering of hyaline cartilage

The method of constructing cartilage tissue from bone marrow-derived cells in vitro is considered a valuable technique for hyaline cartilage regenerative medicine. Using a rotating wall vessel (RWV) bioreactor originally developed to simulate a microgravity environment, we attempted to efficiently construct hyaline cartilage tissue from human bone marrow-derived cells without using a scaffold (Sakai et al., 2009). Bone marrow aspirates were taken from the iliac crests of 9 patients using an 11G Bone Marrow Harvest Needle during orthopaedic surgery (mean age of 36 years, range of 23-62 years; Table 1) as shown in Fig. 6. Briefly, 10 ml of bone marrow sample with anticoagulant was mixed with 25 ml of phosphate-buffered saline, and 35 ml aliquots of bone marrow suspension were overlaid onto a poly-sucrose gradient and centrifuged at 1500 \( g \) for 15 min at room temperature. The cell layer was carefully removed and suspended and cultured in a growth medium. The growth medium consisted of Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Approximately 2-3 weeks later, confluent cells were subcultured and seeded on two representative culture systems (RWV culture and pellet culture) for chondrogenic differentiation using chondrogenic differentiation medium (described in section 2.2).

Table 1. Summary of data from 9 samples (Sakai et al. 2009)

| Donor No | Age (years) | sex | predisposing factor | Nucleated cells in Peripheral blood (×100000 cells/μl) | Nucleated cells in Bone marrow blood (×100000 cells/μl) |
|----------|-------------|-----|---------------------|------------------------------------------------------|-------------------------------------------------------|
| Donor 30 | 28          | Male| Posttraumatic osteonecrosis of the femoral head | 3.8                                                  | 27.5                                                  |
| Donor 31 | 23          | Male| Posttraumatic osteonecrosis of the femoral head | 8.1                                                  | 74.1                                                  |
| Donor 32 | 26          | Female| Posttraumatic osteonecrosis of the femoral head | 10.7                                                 | 22.1                                                  |
| Donor 33 | 26          | Male| Idiopathic osteonecrosis of the femoral head | 7.1                                                  | 36.2                                                  |
| Donor 35 | 42          | Female| Osteoarthritis of hip joint | 7.7                                                  | 32.3                                                  |
| Donor 36 | 37          | Female| Steroid-induced osteonecrosis of the femoral head, SLE | 8.5                                                  | 16.2                                                  |
| Donor 37 | 36          | Female| Steroid-induced osteonecrosis of the femoral head, SLE | 9.9                                                  | 28.3                                                  |
| Donor 38 | 53          | Female| Posttraumatic osteonecrosis of the femoral head | 4.9                                                  | 18.0                                                  |
| Donor 39 | 62          | Female| Idiopathic osteonecrosis of the femoral head | 4.2                                                  | 12.6                                                  |
cartilage tissue from adult human bone marrow-derived cells including mesenchymal stem cells by rotating three-dimensional dynamic culture. After 2 weeks of differentiation and induction of adult human bone marrow-derived cells to form cartilage, the two culture methods were compared using the RWV bioreactor and the pellet culture as shown in Figs 7 and 8. The RWV system produced a larger tissue rich in GAG and collagen II, which are specific components of the cellular matrix of hyaline cartilage. These results suggest that the culture under hydrodynamic conditions using the RWV bioreactor provides a more suitable environment for the induction of cartilage differentiation from adult human bone marrow-derived cells than a conventional static culture system (pellet culture).

Fig. 6. Brief schematic of experimental protocol (Sakai et al., 2009)

In RWV cultures, rotating the culture medium in the vessel provides an ideal three-dimensional system to achieve high-density cell agglutination and obtain a spatially extended extracellular matrix. Decreased shear stress due to the rotating culture fluid makes high-density cell agglutination possible and affords effective nutrition supply and excretion owing to the circulating fluid. As a result, this system provides high tissue production and high maintenance. Cells tend to retain their differentiated phenotype in vitro only if cultured under conditions that resemble their natural environment in vivo. Therefore, it is
reasonable to think that the tissue engineering of cartilage must be conducted in a physiological and mechanical environment similar to that during the *in vivo* ontogeny of the embryo. The formation of cartilage starts with cell agglutination, after early agglutination, an important event for maturation and skeletal patterning (Hall et al., 1992). The formation of bone and cartilage in the fetus occurs in a mechanical environment very similar to an environment with microgravity because the amniotic fluid provides buoyancy to the cells. Many studies have shown the effects of gravity and mechanical load on tissue, and a microgravity environment has considerable effect on tissue formation (Klement et al., 1994, 2004; Freed et al., 1999). Furthermore, some studies have shown an effect of gravity on embryonic bone and cartilage formation. It has been confirmed that the gravity load on mesenchymal stem cells during the early embryonic stages is an important factor determining the orientation of differentiation, and that the effect of a microgravity environment on cells differs between stages; however, the effects of microgravity on the differentiation of mesenchymal stem cells into bone, cartilage and adipose tissue remain to be determined. From the results of this study, the pseudo-microgravity environment provided by the RWV bioreactor may facilitate the differentiation of mesenchymal stem cells into cartilage.

![Fig. 7. (A) Comparison of the tissue formed in the pellet culture and RWV culture. Macroscopic photograph after 14 days of pellet culture (left), using an RWV bioreactor (right). (b) Wet weight of the tissue construct after 14 days. Data are expressed as the mean SD (n=9). : p<0.05 (Sakai et al., 2009)](www.intechopen.com)
Fig. 8. Safranin-O/fast green staining of cell aggregates cultured using an RWV bioreactor (A, B) and pellet culture system (C). (Original magnification x40). Cell aggregates from 3 donors showed distinct staining with safranin-O (A). Cell aggregates from 6 donors cultured using an RWV bioreactor showed partial staining with safranin-O (B). Construct cultured using the pellet culture system showed faint and heterogeneous staining (C). (Sakai et al. 2009)

4. Automatic rotation control system for RWV bioreactors

The RWV bioreactor proved quite useful for the three-dimensional culture of mesenchymal cells as described above. This system is expected to be of laboratory use as well as clinical use, however, it currently requires manual control of the rotation speed to match the increase of mass of the growing tissue as shown in Fig. 2. To overcome this inconvenience, we developed an automatic rotation control system for the RWV bioreactor as shown in Fig. 9. A method of catching the position of the cell aggregates in the rotating vessel is key.

Fig. 9. Automatic Rotation Control System for RWV Bioreactors (for laboratory use)

An image of the vessel containing a medium and a cell aggregate is shown in Fig. 8. As commercially available culture mediums are colored pink or red, it is difficult to visualize aggregates in a rotating vessel equipped with valves etc. The complex structure of the vessel
prevent simple visualization of the cell aggregate with a CCD camera. Consequently, a tricky method was developed using a dual illumination system as shown in Fig. 10. The tissue growing in the RWV vessel was observed using the CCD camera in front of the vessel, and visualized by two illumination systems, cold blue light and white light at the back and front of the vessel respectively, which enabled the visualization of only the tissue. By regulating the RGB signals from the CCD camera, it would be possible to get a masked image of the growing tissue. The control system calculates the position of the tissue and sends a feedback signal to adjust the rotation speed. This automatic system frees up technicians and students.

Fig. 10. Principle of visualization of a tissue in RWV vessel: The visualized tissue is judged by the program as to whether it is in the preset rectangular area in the vessel or not, and the control system sends a feedback signal to the rotation system in the RWV bioreactor to increase or decrease the rotation speed if it is outside of this area

5. GMP grade automatic RWV cell culture system

For using an RWV bioreactor, for instance for cartilage regeneration therapy, a rough scheme of a series of procedures could be drawn as shown in Fig. 11. Bone marrow-derived cells aspirated from the iliac crest are cultured two dimensionally for expansion then three dimensionally by RWV bioreactor, after while they are transplanted into the damaged area. The procedures for cell treatment and cell culture are core processes to produce engineered tissues with structural integrity and functionality. Strict measures to prevent contamination and human error are needed due to the direct use of unsterile products and laborious nature of culture operations. For ensuring a reliable process and good quality products (engineered cartilage), a processing system for RWV culture is necessary and was developed, in which an automatic rotation control (described in the last section) and an automatic medium exchange process are available under GMP level as shown in Fig. 12. The automatic RWV
cell culture system mainly consists of three blocks, a CO$_2$ incubator and two refrigerators. The CO$_2$ incubator is equipped with an RWV system which rotates two vessels independently, stops their rotation when a change of medium is necessary, and moves the vessels outside of the incubator, where two robot arms grasp two syringes from the refrigerators (one with fresh medium from the refrigerator beneath the incubator, the other for wasted medium from the refrigerator on the incubator) and moves them to the top and bottom of the vessel, inserts their needles into the caps of the vessel and changes the medium (Fig.13). After the medium exchange procedure, the robot arms deliver each syringe to each refrigerator, the vessel moves to the normal position and the rotation culture starts. In the rotation culture, the rotation speed is controlled by the method described in the last section.

Fig. 11. Procedure for cartilage regeneration therapy using an RWV bioreactor and bone marrow cells

This system limits its function in rotation culture, however, such automatic processing systems are generally inevitable for safety processing for future therapeutic applications in tissue engineering and cell therapy (Kino-oka et al., 2009).

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Fig. 12. Automatic RWV Cell Culture System

Fig. 13. Two vessels in the CO₂ incubator in the automatic RWV culture system (left) and the vessel out of the incubator with two attached syringes (right)

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