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Studies of Chondrogenesis in Rotating Systems

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Abstract A great deal of energy has been exerted over the years researching methods for regenerating and repairing bone and cartilage. Several techniques, especially bone implants and grafts, show great promise for providing a remedy for many skeletal disorders and chondrodystrophies. The bioreactor (rotating-wall vessel, RWV) is a cell culture system that creates a nurturing environment conducive to cell aggregation. Chondrocyte cultures have been studied as implants for repair and replacement of damaged and missing bone and cartilage since 1965 [Chesterman and Smith, J Bone Joint Surg 50B:184–197, 1965]. The ability to use large, tissue-like cartilage aggregates grown in the RWV would be of great clinical significance in treating skeletal disorders. In addition, the RWV may provide a superior method for studying chondrogenesis and chondrogenic mutations. Because the RWV is also reported to simulate many of the conditions of microgravity it is a very useful ground-based tool for studying how cell systems will react to microgravity.

Key words: bioreactor, chondrocytes, cartilage, bone, gravity

Cartilage differentiation and endochondral bone formation are events in a developmental cascade that begins with the formation of the cartilaginous skeleton of the embryo and ends, in mammals, with the post pubertal closing of the growth plates [Urist, 1983]. Bone has remarkable regenerative powers: the entire sequence of endochondral ossification may be reactivated if necessary for healing of fractured bones [Urist, 1983]. The control of the process is complex, involving local and systemic factors, and including biomechanical parameters [Urist, 1983]. Dissection of the mechanism regulating chondrogenic development and endochondral ossification has enormous clinical significance: skeletal disorders—inherited or induced—cost billions of dollars a year to treat, and cause untold suffering and heartache. Osteoporosis, osteopetrosis, craniofacial anomalies, fracture healing (particularly of the elderly), and orthopedic and dental implants are all subjects of intense basic and applied research in an effort to mold, replace or repair bone [Glimcher and Lian, 1988]. Chesterman and Smith began studying chondrocyte cultures as implants to repair and replace damaged or missing bone and cartilage as early as 1965 [Chesterman and Smith, 1965]. The use of the rotating-wall vessel (RWV) at Johnson Space Center promises to be an invaluable tool for the growth of large, healthy aggregates for studies of regulation of differentiation or for implantation into bony defects.

A BRIEF HISTORY OF BONE INDUCTION

Chondrogenic induction for bone repair and replacement has as its goal the production of new bone through endochondral ossification. Biologic approaches for repair or replacement of cartilage and bone provide a significant advantage over the use of artificial materials; they become integral parts of the tissue into which they are implanted [Nevo et al., 1991]. Repair of articular cartilage by tissue implantation is of major importance, since cartilage does not have the reparative capabilities of bone. The focus of this paper, however, will be on systems used to repair bone.

One of the best-studied systems for repair of bony defects is the matrix induced bone system developed by Urist and Reddi in which particles of demineralized bone matrix induce bone when implanted into ectopic sites in rats [Reddi et al., 1987; Urist, 1983]. Recently, osteogenin (one of the active factors in bone matrix) derived from

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cadaver bone, and demineralized bone matrix were used to induce formation of trabecular bone from muscle flap tissue placed in a mold of particular shape [Khouri et al., 1991]. The tissue stages involved in this inductive process—chemotaxis, mitosis, differentiation of cartilage and its calcification, vascular invasion, cartilage resorption, bone differentiation, and hematopoiesis—are very similar to stages of endochondral ossification in vivo and ossicles have been used to study that process [Reddi et al., 1989].

The demineralized bone matrix system has many advantages for implantology—there is no difficulty in obtaining sufficient amounts of material because animal or human cadaver bone is used. Also, the demineralization process eliminates much of the immunogenicity of the implant, and since the host's own tissues are induced to form cartilage, there is no possibility of rejection. But one must still wonder, why wait a week or more to generate cartilage in an implant? Why not just start with cartilage in the first place? Such studies have been done, but success has been limited [Nevo et al., 1991]. In allogenic grafts, an immune response is evoked that destroys the graft. Autogenic grafts have little success because of the limited regenerative ability of adult cartilage. The use of aggregated embryonic limb cells is a viable alternative that has not been adequately investigated [Nevo et al., 1991].

CARTILAGE DIFFERENTIATION IN THE EMBRYONIC LIMB

Limb buds first appear as outgrowths from the flanks of the embryo, consisting of mesenchymal cells covered by an ectodermal layer [Thieler, 1972]. At this time, the cells have not yet been determined, and their fate (muscle, cartilage, etc.) can be altered by transplanting them to another area of the limb [Searls and Janners, 1969]. The first sign of the developing skeleton within the limb bud is the appearance of precartilaginous blastemata produced by aggregation of the mesenchymal cells [Rugh, 1968]. This aggregation was deemed to be a necessary step in chondrogenesis, on the basis of in vitro studies that found that limb mesenchymal cells formed cartilage when cultured at high densities [Lavietes, 1970]. Electron microscope studies showed that during aggregation, cells made extensive cell-cell contacts, just prior to beginning their production of matrix, which is accompanied by changes in cell ultrastructure and shape [Thorogood and Hinchliffe, 1975]. Later studies however, demonstrated that cell-cell contact is not necessarily required for differentiation; limb mesenchymal cells grown in the presence of cytochalasin D round up and differentiate into cartilage [Archer et al., 1982; Zanetti and Solursh, 1984]. As the cells aggregate (or round up) in vivo or in vitro, there is an increase in levels of cyclic AMP [Elmer et al., 1981]. Associated with this increase are gene activation and production of RNA for various matrix molecules specific to cartilage, such as collagen type II and X, and cartilage core protein [Oshima et al., 1989]. Protein production with appropriate intercellular processing and glycosylation follows [Dorfman, 1981]. Aggregation and secretion of collagen and proteoglycans follow, and additional aggregation of matrix, including collagen proteoglycan interactions, takes place in the extracellular environment [Trelstad and Silver, 1981]. (Some evidence for the cosecretion of collagen and proteoglycans exists however, showing that these important interactions can take place prior to secretion [Landis and Hodgens, 1990].) Mineralization of the cartilage matrix then occurs, by a process that is as yet unknown [Hunter, 1991] and then calcified cartilage is vascularized leading to the removal of the cartilage and its replacement by bone [McFarland et al., 1990; Trueta, 1963].

The ends of the long bones remain cartilaginous and provide for rapid longitudinal growth through continuing endochondral ossification.

ENVIRONMENTAL FACTORS AFFECTING CARTILAGE DIFFERENTIATION

Oxygen Tension

Cartilage is an avascular tissue, and oxygen tension in both the embryonic limb and the developing growth plate is thought to be negatively correlated with vascularity. In the embryonic limb, the division between developing myogenic tissue and the cartilage anlage is delineated by the vascular system [Caplan and Koutroupas, 1973]. In the growth plate, lack of oxygen in the hypertrophic zone is deemed necessary for a hypoxia-related modulation of chondrocyte metabolism that results in calcification [Shapiro et al., 1983]. Low oxygen tension (5%) in cultures of embryonic limb mesenchymal cells potentiates expression of the chondrogenic phenotype, while myogenic expression is potentiated by high oxygen tension [Caplan and Koutroupas, 1973; Pawelek, 1969]. To our knowledge, the only

studies of the response of cartilage to *no* gas exchange were performed in this author's laboratory during testing of hardware used in growing micromass cultures of embryonic limb cells aboard the shuttle [Duke et al., 1989, 1991]. (This experiment, known as "CELLS," flew on the shuttle *Discovery* in January 1992—see Micromass Cultures and the CELLS Experiment.) This hardware design was a closed system with a piston, while a later design had a perforated piston to allow for gas exchange and the final design used a gas permeable silicone membrane. Studies with the non-perforated piston hardware found that cartilage developed earlier (more on an in vivo time scale) than in the hardware with perforated pistons (Duke et al., unpublished observations).

Growth Factors

Cartilage differentiation is affected by numerous growth factors. Some of these identified so far are growth hormone [Isaksson et al., 1982], somatomedin [Elders et al., 1975], cortisone [Von der Mark and Von der Mark, 1977], insulin [Unger et al., 1991], epidermal growth factor [Hiraki et al., 1990], transforming growth factor β [Rosier et al., 1989], vitamin D metabolites [Burch et al., 1988], and insulin-like growth factors [Sessions et al., 1991]. The mechanism of action of these factors, and their interactions remain largely unknown.

Cell-Cell, Cell-Matrix, and Matrix-Matrix Interactions

Comparisons of chondrogenesis in mutations involving the skeleton or as a result of exposure to teratogens have found that rather small changes in cell-cell interactions or matrix production and aggregation can have severe, even lethal consequences [Trelstad and Silver, 1981].

Biomechanical Factors

Biomechanical factors are extremely important in cartilage differentiation, as shown most dramatically by the distraction method of bone lengthening [Turner and Anderson, 1991]. In this technique, the long bone is fractured and the ends are slowly pulled apart (distracted) at a steady rate. Cartilage formation is stimulated between the fracture ends, and undergoes the normal endochondral ossification process. In vitro, mechanical stimulation of micromass cultures has been shown to increase matrix production [Veldhuijzen et al., 1987].

HISTOTYPIC AGGREGATION IN STUDIES OF LIMB DEVELOPMENT

Studies of cell aggregation and cell sorting began with E.B. Wilson's observations of species specific sorting between members of the sponges [Wilson, 1907]. Holtfreter's studies of dissociated and reaggregated embryos also showed that various tissues aggregated together and resumed their normal positions within the reconstructed embryos [Townes and Holtfreter, 1955]. Later, Moscona extended these studies by trypsinizing tissue and reaggregating it in rotary systems [Moscona, 1962]. These systems were extremely useful for studies of cell adhesiveness and cell sorting, and were used to identify defects in these processes in various skeletal mutants, including brachypodism [Duke and Elmer, 1977, 1978, 1979].

The brachypod mutation is an autosomal recessive mutation expressed during the condensation stage of limb development which affects aggregation within the blastemata [Milaire, 1965; Gruneberg and Lee, 1973]. Concanavalin A studies show that there is a decline in reactivity with Con A in normal cells between days 11 and 13 of development, but which is delayed by 24 hours in the mutant [Hewitt and Elmer, 1976].

Because the anomaly is first apparent at 12 days of gestation, 12 day embryonic mouse limb buds were used for reaggregation studies of cellular adhesiveness [Duke and Elmer, 1977]. Dissociated cells in 3 ml of medium were placed in 10 ml Erlenmeyer flasks at a concentration of 1×10^6/ml and rotated at 70 rpm at 37°C. Cell counts were made every 15 min until the number of single cells remained constant. The number of single cells declined more rapidly in mutant cultures, indicating that these cells are more adhesive than normal cells [Duke and Elmer, 1977]. Chondrogenesis within the brachypod aggregates was also delayed, and the extent of chondrogenesis over a 7 day culture period was much less than in normals [Duke and Elmer, 1977].

Sorting of cells in mixtures of normal and mutant cells (one or the other having a radioactive label) were not successful (Duke, unpublished observations) due probably to the adhesive difference not being large enough, so aggregate fusion was used to additionally assess adhesiveness [Duke and Elmer, 1978]. In these studies, aggregates constructed in rotary culture were

allowed to fuse in a hanging drop (again with one or the other being labeled), then fixed and sectioned. After autoradiography, the interface between the two tissue types was examined to determined the more adhesive tissue—defined by Steinberg as that tissue which will move to the center of the aggregate [Steinberg, 1970]. The fragment fusion studies clearly showed that brachypod cells have greater adhesiveness [Duke and Elmer, 1978].

Observations on adhesiveness were confirmed in electron microscope studies, which show that brachypod cells have more extensive areas of cell-cell contact than do normal cells at 12 days of development (postaxial) and that these contacts persist longer in the mutant, and contribute to the delay in proteoglycan production which is in turn related to the extremely thick collagen fibers produced by the mutant [Duke and Elmer, 1979].

CARTILAGE IN ALTERED GRAVITY

Skeletal development including remodeling and repair is of particular interest to NASA: all components of the skeletal system studied to date show that the skeleton is profoundly affected by gravitational changes. Bone, cartilage, and ligaments of spaceflown rats have shown significantly altered differentiation [Duke et al., 1990, 1991; Morey-Holton and Cone, 1990; S. Doty, personal communication] and fracture healing is virtually nil [Kaplansky et al., 1991]. The bone demineralization and negative calcium balance observed in astronauts and cosmonauts remain a concern and may be a limiting factor in the ability of humans to live in space [Tilton et al., 1980]. Cartilage differentiation is equally important because of its involvement in formation of the embryonic skeleton, in growth of the long bones and in fracture healing [Urist, 1983].

Exposure to altered gravity alters differentiation of cartilage in mouse embryonic limbs and rat growth plates as evidenced in the following examples:

1. Early ossification of femoral heads of centrifuged rats [Smith, 1975].
2. Delayed mineralization and abnormal collagen formation in growth plates of rats flown on Cosmos 1129 [Matthews, 1981].
3. Suppression of morphogenesis in embryonic mouse limbs exposed in vitro to 2.6 g due to precocious chondrogenesis [Duke, 1983].
4. Changes in height, cell number, and extracellular matrix (altered collagen fibril length and width, and PGGs per unit area) in tibial epiphyseal growth plates of rats flown on *Spacelab 3, Cosmos 1887,* and *Cosmos 2044* [Duke et al., 1990; Montufar-Solis et al., 1991].
5. Altered matrix vesicle distribution, and different pattern of PGG size within tibial epiphyseal growth plates of *Spacelab 3* rats [Duke and Montufar-Solis, 1989].

This is not surprising, since the response of cartilage to changes in loading is a fundamental principle of orthopedics [Turner and Anderson, 1991]. Systemic factors are also involved: decreased growth hormone production in rats [Hymer et al., 1985] certainly contributes to the changes in in vivo differentiation in space. Some of the changes seen in our studies, particularly in matrix production and aggregation, resemble changes seen in various chondrodystrophies [Duke and Montufar-Solis, 1989; Duke et al., 1990].

Our studies of epiphyseal cartilage have been primarily morphometric, due to experimental restrictions, and inconclusive because of the lack of in-flight animal sacrifice, small sample number, and the many variables of spaceflight, including the inherent complexities of whole animal systems and problems that are technologically impossible to control to date [Duke et al., 1990]. In addition, studies using epiphyseal cartilage must contend with the presence of cells in all stages of the chondrogenic process, as well as in steps of endochondral bone formation [Reddi et al., 1989]. In vitro systems offer the advantage of a larger sample number, in-flight fixation, and the ability to more accurately control the cell's environment.

Micromass Cultures and the CELLS Experiment

Events of cartilage differentiation are duplicated to a large extent by the use of high density micromass cultures. High density culture favors the differentiation of chondrocytes, whereas at lower cell densities, other cell types may predominate [Ahrens et al., 1977, 1979; Umansky, 1966]. Chondrogenesis in micromass cultures has been very well studied and found to be similar to that occurring in vivo. During the first 24 hours, the cells divide and then begin to form small aggregates which are the in vitro correlates of the precartilaginous blastemata of the limb. By 48 hours, cells within some aggregates

begin producing cartilage matrix (chondroitin sulfate and Type II collagen), and the aggregates (now called nodules) stain positively with Alcian blue [Ahrens et al., 1977]. Cells go on to produce collagen Type X which is associated with cell hypertrophy [Zanetti et al., 1990]. Mineralization of this matrix can also occur [Olsen, 1981], although vascularization requires addition of the appropriate components of the blood forming system [McFarland et al., 1990]. Many of these same steps occur in 3-dimensional cartilage aggregates [Duke and Elmer, 1977, 1978].

Micromass cultures were flown on the space shuttle *Discovery* in January 1992 as part of the International Microgravity Laboratory-1. This experiment, known as CELLS, was designed to study chondrogenesis in microgravity. The experiment contained four groups of cell cultures, a flight set at microgravity, a flight set on a 1*g* centrifuge, a ground set at 1*g*, and a ground set on a 1.4*g* centrifuge. Medium was changed daily for 5 days and cultures from each group were fixed at the medium change.

Alcian blue staining for cartilage specific proteins of the flight and ground samples showed the cultures were viable and had differentiated into chondrocytes producing cell aggregates and nodules, although in many of the microgravity cultures, the cell membranes are much smoother than controls and cells lack the obvious matrix that surround the cells in ground cultures. Several unusual formations exist in the flight cultures, both microgravity and 1*g* (centrifuged). These may be highly ruffled areas of cell membrane, peeling of the cell monolayer, or crystallized calcium salts [Duke et al., 1992].

Observations of the flight cultures using scanning electron microscopy and light microscopy are supported by higher magnification transmission electron micrographs. If the final conclusion from this flight experiment is that microgravity significantly alters chondrogenesis, many more studies will have to be done to determine which processes are being affected and what techniques may compensate for the changes. The RWV will be a very useful tool to examine cell systems on earth under conditions similar to microgravity.

Bioreactor Results

The precision tissue culture devices developed by NASA, called rotating-wall vessels (RWVs), have advantages such as homogeneous distribution of cells, nutrients, and waste products, with less damaging turbulence and shear forces than conventional bioreactors [Tsao et al., 1992; Goodwin et al., 1992]. Cultures under such conditions have higher growth rates, viability, and longevity, which allow larger "tissue-like" aggregates to form than in environments with high fluid velocity gradients and sedimentation. Embryonic limb mesenchymal cells grown on microcarriers in the RWV at Johnson Space Center were shown by cartilage specific Alcian blue staining to have differentiated into matrix-producing chondrocytes [Daane et al., 1991]. The cells and microcarriers aggregated during the experiment and the aggregates continued to increase in size over the 65-day culture period.

"Ruffled or peeled" areas seen in the scanning electron micrographs of the cells grown in the RWV were remarkably similar to areas observed in the CELLS flight experiment scanning electron micrographs. These characteristics have not been previously seen in chondrocyte micromass cultures grown at 1*g* in tissue culture plates or in flight hardware. This may give further support to the argument that the RWV offers similar conditions to those experienced in microgravity. The size and apparent good health of the cell aggregates grown in the RWV also advocates use of the RWV for growing cells to be studied for possible use in implants.

IMPLICATIONS FOR THE FUTURE

The rotating system provides a three-dimensional aspect not seen in most other culture systems used and a tissue homogeneity not possible with explants of limbs or limb elements. Chondrogenesis in the aggregates from the RWV's more closely resembles chondrogenesis in vivo than conventional cell culture methods using monolayers [Daane et al., 1991]. Because defects arising from mutations or teratogens are expressed in 3-D aggregates produced in vitro [Duke and Elmer, 1977, 1978], the RWV aggregates may provide a superior system for studying and correcting defects and growing healthy tissue for implants. The RWV system is reported to simulate many characteristics of microgravity [Schwarz et al., 1991] and it may be that the cartilage produced by the system will be abnormal since microgravity has been shown to alter cartilage differentiation [Duke et al., 1990; Montufar-Solis et al., 1991]. If this is the case, then the RWV provides an opportunity using a relatively simple, low maintenance system to study changes produced in microgravity and to

perhaps find methods for countering the effects incurred by exposure to microgravity.

STUDIES OF CHONDROGENIC REGULATION

The large (1 cm) cartilaginous aggregates produced in the RWV may be useful in basic studies of chondrogenic regulation. Just as skeletal abnormalities due to mutation or chemical teratogens assist in dissecting how skeletogenesis is regulated, the use of gravity—a physical teratogen—can provide insight into cartilage regulatory mechanisms [Kochar, 1985]. The rotating systems at the Johnson Space Center are reported to simulate some aspects of microgravity [Schwarz et al., 1991], and since chondrogenesis in microgravity is affected in vivo [Duke et al., 1990; Montufar-Solis et al., 1991], and in vitro [Daane et al., 1991] chondrogenesis within aggregates formed in rotary systems may show similar changes in differentiation, and in production and aggregation of matrix. If deleterious changes are found in the aggregates, growth factors can be used in attempts to correct these defects [Hiraki et al., 1990], and to thus clarify the role of these factors in cartilage differentiation. It is quite likely that growth factors do not interact the same with a three-dimensional culture system as in a more conventional culture plate system [Lewis and Hughes-Fulford, 1991].

No studies of the effects of very low O_2 concentration ($< 5\%$) have been carried out on cartilage despite its avascularity and the presumed role of hypoxia in cartilage differentiation. Our preliminary studies using the original CELLS hardware with no gas exchange found enhanced chondrogenesis, but the precise regulation of oxygen concentration in conventional cultures systems is difficult, if not impossible. These studies can easily be carried out in the RWV.

Rotating systems have been used for the culture of bone cells, but reports of mineralization of the cells are scanty, consisting of unpublished TEMs [Cone et al., 1989; Lewis and Hughes-Fulford, 1991]. For studies of cartilage mineralization, we plan to examine mineral, not only by ultrastructure, but also by microprobe and X-ray diffraction. Mineralization in three-dimensional aggregates may be more representative of mineralization in vivo than is mineralization in a two-dimensional culture.

TOPOBIOLOGICAL STUDIES

Spaceflight studies indicate that cell-cell interactions—so important in developmental processes—are affected by the space environment [Duke and Montufar-Solis, in press]. Cell-cell interactions and differential adhesiveness can be studied in the bioreactor just as they have been in other rotating systems [Steinberg, 1970; Duke and Elmer, 1977]. Studies of the appearance of adhesive molecules, and their location can also be carried out [Edelman, 1983].

IMPLANTATION POSSIBILITIES

In order to use cultured chondrocytes for implants, several questions must be kept in mind [Nevo et al., 1991]:

Is there enough tissue to fill the defect?
How can the graft be shaped?
How can immunogenicity be reduced?
Can the cartilage phenotype be retained during culture?
Is a bioactive, biodegradable carrier available?

The use of embryonic cells cultured in the RWV seems to be the answer to these questions, at least theoretically. Aggregates of embryonic tissue grown in the RWV continued to increase in size during the lengthy culture period, in contrast to other culture systems in which cell division stops upon confluency [Umansky, 1966]. At 1*g*, the limiting size of the aggregates is about 3 mm; additional growth results in the aggregates tumbling within the RWV.

Embryonic cells are less immunogenic than adult cartilage cells, and culturing them further reduces immunogenicity. This is because culture, even for a brief time, allows the regeneration of matrix components that may have been stripped from the cells during the isolation process and matrix is much less immunogenic than is the cell's bare surface [Nevo et al., 1991]. Also, the presence of a perichondrium reduces immunogenicity, and cartilage aggregates have a perichondrial-like layer [Duke and Elmer, 1977]. With enough reduction in immunogenicity, xenogeneic grafts may be possible, eliminating the need for human tissues to be the source of grafts for humans [Nevo et al., 1991].

Implantology also requires a material that can be used to adhere the graft to the host tissue [Nevo et al., 1991]. In our preliminary studies for the cells experiment, we used a number of substrates, and found that Cell-Tak® (Collaborative Research, Inc.), a biologic adhesive derived from mussels, worked exceptionally well. This has also been used in implant studies [Grande and Pitman, 1988].

Repair of the defect will depend, in part, on the ability of the aggregate to mineralize and vascularize [Trueta, 1963]. Instead of implanting cartilage into host animals, or using a coculture system to assess the ability of the aggregate to be vascularized, it should be possible to assay for endothelial cell stimulating angiogenesis factor (ESAF) as a measure of the vascularization potential of the mineralized cartilage aggregate [Brown et al., 1987]. ESAF is produced by hypertrophic chondrocytes, when stimulated by the proximity of crystals in the matrix [McFarland et al., 1990]. The release of this potent activator of collagenolysis is part of the angiogenic cascade operating during endochondral ossification [Brown and Weiss, 1988].

BIOREACTORS AND SPACE

The culture environment provided by the RWV is reported to be similar in many aspects to microgravity, but results in the RWV at 1*g* have yet to be compared to results from flight experiments. This comparison cannot be made accurately for cartilage until cartilage is flown in such a system in space. Chondrogenesis in space has been studied primarily in growth plates of spaceflown rats and only superficial comparisons to aggregates of embryonic cells grown in the RWV can be made. Similarly, parameters involved in the CELLS micromass experiment (cells on a substrate; mechanical perturbances; lack of mixing) make valid comparisons difficult. The parameters of chondrogenesis that the rotating system as a microgravity simulator actually simulates must be determined for establishment of its validity for ground-based studies and/or a microgravity predictor.

In space, where lower shear and turbulence in the cultures is possible [Tsao et al., 1992] and where the aggregates can remain in suspension, formation of larger aggregates is predicted. These aggregates must be characterized as to normality of chondrogenesis, and their capacity for mineralization and vascularization has to be assessed. The production of larger aggregates will provide more tissue for chondrogenic studies and for repair of larger defects.

Since the normality of these aggregates must be established as well, the influence of microgravity on chondrogenic regulation will of necessity be addressed. The parameters of chondrogenesis that the rotating system as a microgravity simulator actually simulates will be determined for establishment of its validity for ground-based studies and/or as a microgravity predictor.

CONCLUSIONS

Cartilage aggregation is not a new technique, but previous studies of such aggregates were primarily histological and were used to study differential adhesiveness, rather than genomic regulation. The latter has been carried out primarily in various cell culture systems [Glimcher and Lian, 1989]. The use of the RWV makes it possible to apply modern techniques of studying gene expression to 3-D cartilage aggregates formed in a rotating system. The use of aggregates of embryonic cartilage to heal bony defects has not been widely studied, possibly because aggregates have been simply too small to be useful. The paradigm, with few exceptions, has certainly been that bone or bone products are used to induce bone and cartilage to repair/replace cartilage [Nevo et al., 1991]. Also, the tantalus of the bone induction system and bone morphogenic protein has influenced many studies in this area. The use of the RWV to produce cartilage for implants is an exciting and promising development.

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