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Growing Tissues in Real and Simulated Microgravity: New Methods for Tissue Engineering

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Tissue engineering in simulated (s-) and real microgravity (r-μ*g*) is currently a topic in Space medicine contributing to biomedical sciences and their applications on Earth. The principal aim of this review is to highlight the advances and accomplishments in the field of tissue engineering that could be achieved by culturing cells in Space or by devices created to simulate microgravity on Earth. Understanding the biology of three-dimensional (3D) multicellular structures is very important for a more complete appreciation of *in vivo* tissue function and advancing *in vitro* tissue engineering efforts. Various cells exposed to r-μ*g* in Space or to s-μ*g* created by a random positioning machine, a 2D-clinostat, or a rotating wall vessel bioreactor grew in the form of 3D tissues. Hence, these methods represent a new strategy for tissue engineering of a variety of tissues, such as regenerated cartilage, artificial vessel constructs, and other organ tissues as well as multicellular cancer spheroids. These aggregates are used to study molecular mechanisms involved in angiogenesis, cancer development, and biology and for pharmacological testing of, for example, chemotherapeutic drugs or inhibitors of neoangiogenesis. Moreover, they are useful for studying multicellular responses in toxicology and radiation biology, or for performing coculture experiments. The future will show whether these tissue-engineered constructs can be used for medical transplantations. Unveiling the mechanisms of microgravity-dependent molecular and cellular changes is an up-to-date requirement for improving Space medicine and developing new treatment strategies that can be translated to *in vivo* models while reducing the use of laboratory animals.

Introduction

A LONG-TERM SPACEFLIGHT induces a variety of health problems, such as cardiovascular and immune disorders, osteoporosis, muscle atrophy, disruption of the biological clock, and others.[1–7] For a long time, it has been clear that microgravity induces alterations in human cells.[8–10] However, the corresponding mechanisms are still unknown.

Spaceflight missions are very rare and costly. Therefore, researchers have developed techniques to simulate microgravity on Earth and to prepare their future Space missions.[11–14] Several cell culture technologies simulating microgravity can be applied in tissue engineering and serve as ground-based facilities for biomedical research. It has been shown that gravitational unloading induces three-dimensional (3D) growth and assembly of cells into functional tissues.[15]

Weightlessness provides a very special environment for the cells because of its lack of sedimentation and convection. Using the fast-rotating 2D clinostat microscope at the German Aerospace Center in Cologne, Germany, we have seen that after 24 h follicular thyroid cancer cells of the FTC-133 cell line start to form 3D aggregates (unpublished data). A similar finding was observed by investigating endothelial cells (ECs) on the random positioning machine (RPM). After 24 h, small, 3D aggregates were detectable.[16] Three-dimensional aggregates of cancer cells have become an important topic in cancer research, because they represent a very simple model of a tumor. Multicellular tumor spheroids (MCTSs) resemble the *in vivo* situation more than monolayer cells, but they are not as complex as natural tumors. They mimic small metastases and areas of solid tumors *in vivo*. Therefore, they

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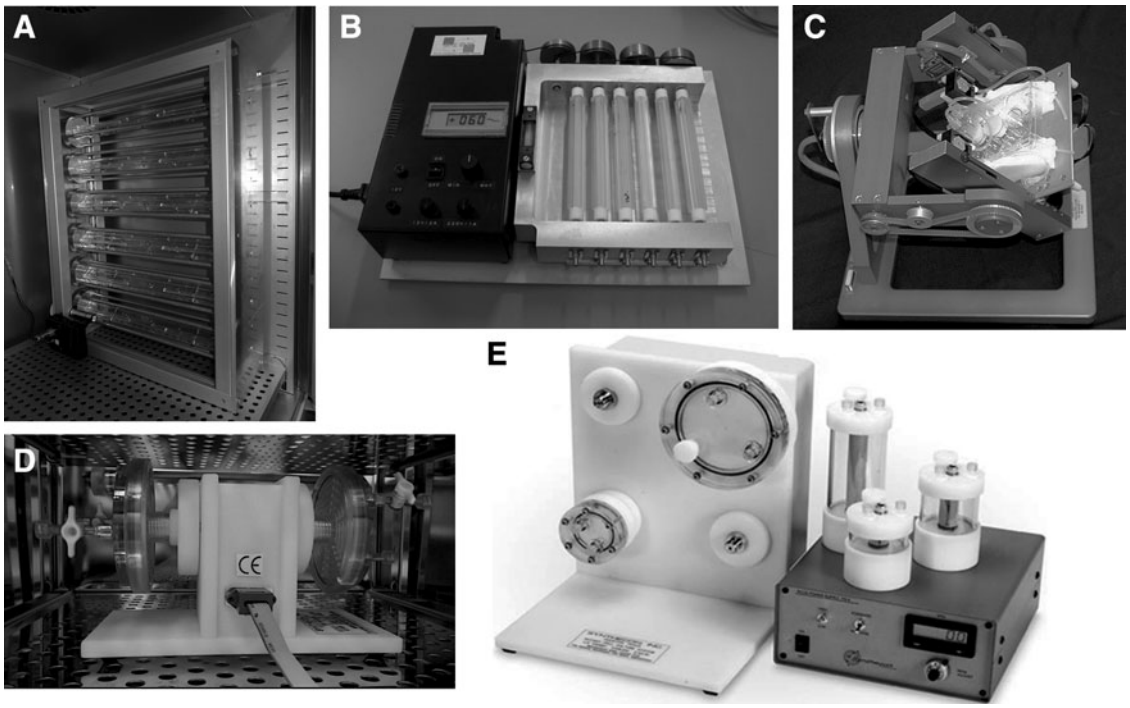

FIG. 1. Devices suitable for tissue engineering. **(A)** Two-dimensional clinostat in an incubator constructed by the German Aerospace Center (DLR), Institute of Aerospace Medicine, Biomedical Science Support Center, Gravitational Biology, Cologne, Germany. **(B)** Example of a fast-rotating 2D clinostat manufactured by CCM (Neunen, The Netherlands). The system holds three static and three rotating tubes of about 10 mL volume. Rotation speed can be adjusted between 30 and 150 rpm. (Image: J. van Loon, DESC, Amsterdam, The Netherlands). **(C)** The desktop random-positioning machine (RPM). In this picture the automated fluid managing system COBRA is mounted on the platform together with a standard 12-well tissue culture plate (Image: Dutch Space, Leiden, The Netherlands). **(D)** Rotating wall vessel (RWV) with two 50-mL vessels in an incubator from Synthecon. **(E)** The rotary cell culture system (RCCS) from Synthecon dedicated for stem cell research with different sized culture vessels (Image from company Web site; Synthecon, Houston, TX).

appeared suitable for developing *in vitro* anticancer drug test systems and can be used for sparing of animal experiments.[17]

In plastic reconstructive surgery small vessels or pieces of tissue are often needed. Therefore, surgeons are looking for alternative sources of tissue. One source is engineered tissues or vessels. If we can go from single cells to 3D tissues, then we should aim as well as be able to construct a transplantable organ or part of an organ in the future. Tissues or vessels can be engineered under weightlessness at low shear stress.[18] They are formed in the absence of an artificial matrix or scaffolds.[10–12] Hence, these types of constructs have the advantage that they are not afflicted with foreign materials.

This review will introduce available devices to simulate microgravity on Earth: the RPM, the rotating wall vessel (RWV), and the clinostat (Fig. 1). We will review the available articles that demonstrate research in the field of tissue engineering under microgravity. Moreover, we will focus on the current knowledge about the involved signaling processes for 3D growth in Space and on Earth and new methods for tissue engineering of intima constructs, cartilage, and thyroid cancer spheroids (Table 1).

Table 1. Summary of Articles Addressing Tissue Engineering Ordered by Tissue

Tissue of	*Experimental goal (Ref.)*
Endothelial cells	3D growth,[12,53,55] altered gene expression,[20,97,105] actin polymerization,[38] increase in apoptosis,[53,77] influence of bFGF,[16] VEGF and bFGF influence,[60,104] influence on caveolae and caveolae constituents,[73] angiogenic response[77]
Cartilage	Characterization of human chondrocytes,[54,109,114,117,118,119] changes in morphology, gene expression, and protein content,[34,110,111] tissue engineering[106–108,112,113,115,116]
Thyroid	Differentiation and increase in apoptosis,[10] spheroid formation,[11,52] neoangiogenesis,[17] thyroid cell growth,[120] interaction of proteins,[128] influence of keratinocyte growth factor,[129] gene expression profile, and altered cytokine secretion[133]
MCSs	Human follicular thyroid carcinoma cells,[10,11,17,52,80,129] prostate cell cultures[79]

MCSs, multicellular spheroids; 3D, three-dimensional; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.

Devices to Generate Simulated Microgravity

Exposing cells to real microgravity (r-μ*g*) is either very expensive or provides time periods of microgravity that last only seconds or minutes, which are too short for tissue engineering studies, although such durations can be useful to explore various intra- and inter-cellular processes under altered gravity conditions.[19–21] Therefore, devices that generate simulated microgravity (s-μ*g*) conditions are most often used for tissue engineering.

Fast-rotating clinostat

Wolfgang Briegleb from the German Aerospace Center (DLR) initiated the construction of fast-rotating clinostat (FRC)[22,23] typically consisting of a pipette-like tube filled with a cell suspension. The tubes have an internal diameter of some millimeters and rotate at a speed between 60 and 90 rpm (Fig. 1A). It is argued that the coupling between the cells and their respective static surrounding liquid boundary layers is the main reason for this microgravity simulation paradigm.[24,25] FRCs (Fig. 1B) are widely used but they have their limitations. A balance between fluid density, viscosity, and cell-specific density is necessary. To have a high quality of microgravity simulation and a minimal residual acceleration, a proper FRC functionality with larger, that is, heavier, samples such as tissue constructs should be guaranteed. Also, a clinostat with its tube diameter of several millimeters has a too limited volume to accommodate a wide range of samples. Tubes cannot be made too wide in order to not increase the centrifugal forces driving the cells to the wall while rotating at higher speeds. This limits studies that require larger samples and volumes as needed in tissue engineering.

Random-positioning machine

A 3D clinostat[26] consists of two frames, each driven by a dedicated motor. In the initial designs the speed of the motors was such that each frame rotated with a constant speed. Later a similar system was developed where the speeds and the direction of rotation of each frame were different and randomized, the so-called RPM[26–29] (Fig. 1C).

In an RPM one could principally work with larger liquid volumes. Cells and larger multicellular constructs are not exposed to a constant, solid body fluid rotation but rather mainly freely and randomly floating within the liquid phase. An initial study demonstrated that such a movement does generate small fluid shear forces at the container wall in the order of 6 mPa (0.06 dyne/cm^2).[30] Cells in an RPM are not only oriented randomly with respect to the gravity vector, but are also more likely to interact with other cells within the experimental volume. Hence, there is an increased opportunity to form multicellular structures. The slight fluid flow around cells and constructs also facilitates an increased provision of nutrients and gasses while waste products are more efficiently removed, compared to static batch cell culture conditions where nutrients are delivered by limited convective flows and the very inefficient process of diffusion.[25] The ample nutrient and gas supply to the samples also permits to work with higher cell densities, again increasing the probabilities of cell–cell interactions. Due to the construction of an RPM a broad variety of sample enclosures are applied in RPM studies. This ranges from regular T25 flasks[31,32] to multiwell plates,[33] flask on slides,[34] or more dedicated devices.[35] Performing RPM studies, where the best simulation of microgravity is in the center of rotation of the two axes, has limits for the size of the preferred volume for the samples. Depending on the speed of rotation and the distance from center, an acceptable residual gravity can be obtained in the order of 10^{-4} *g* by a maximum speed of 60°/s at a radial distance of 10 cm.[25] This allows for a maximum working volume of more than 4 L. Adding drugs or fixing samples during rotation is cumbersome, if not impossible, in an RPM. However, recently tools have been developed to enable fluid managing while rotating.[29] RPMs are commercially available by Mitsubishi Heavy Industries (Kobe, Japan) and Dutch Space (Leiden, The Netherlands), while various academic groups developed similar systems of 3D clinostats and RPMs.[35–40]

Rotating wall vessel

The RWV (Fig. 1D, E) developed by NASA[41] is now commercially available through Synthecon, Inc. (Houston, TX) and is a slow-rotating, relatively large liquid-filled container. It has some overlaps with a 2D clinostat in such a way that cells within the container are prevented from settling by a constant rotation. The rotation speed should be matched to the terminal velocity of the cells, nodules, or other samples within the fluid. The proper rotation speed is mainly determined by the specific weight of the cells, the fluid density, and viscosity, although tissue surface properties and shape also play a role. The cells and tissues in the RWV are constantly falling within the fluid. The settling velocity and direction combined with the rotation of the fluid generated spiral trajectories within the vessel.[42] This motion of the samples relative to the fluid generates fluid shear forces on a particle surface ranging from 180–320 mPa (0.18–0.32 dyne/cm^2) for 50-μm beads[43] and ~500 mPa (0.5 dyne/cm^2) with 3D aggregates of BHK-21 cells[44] to 520–780 mPa (5.2–7.8 dynes/cm^2) for a 200- or 300-μm spherical object.[45] Over the years various models based on the initial RWV have been developed, differing in vessel geometry and aspect ratio and gas supply (perfusion), such as the 250-mL, slow-turning lateral vessel (Synthecon)[46] or the 50-mL high aspect ratio vessel (HARV; Synthecon)[47] or the rotating-wall perfused vessel (Synthecon)[48] (Fig. 1D, E).

Known Mechanisms Involved in Spheroid Formation

In 3D aggregation processes, it does not play a role whether gravity forces are absent during a spaceflight or whether the gravity vector is randomized only by the RPM or the FRC.[2,11,49–51]

Only a part of the cells of a monolayer change their growth behavior, when exposed to microgravity, while the other part continues to grow adherently.[52–54] However, when exposed to microgravity, both the adherently and the 3D growing cells alter their gene expression patterns.[55] Very often the decrease or increase of the protein content found in a cell population under microgravity is accompanied by a change in the number of cells bearing the respective antigen.[10,56] It is interesting to explore whether cellular heterogeneities found in cell lines before exposition to microgravity[57,58] are further expanded under microgravity. In addition, the signals

delivered by removal or randomization of gravity forces cause the formation of different types of aggregates depending on the kind of cells cultured. While cancer cells mainly assemble to spheres,[52] for example, ECs form tubes[8] and chondrocytes form pieces of cartilage,[54] *in vitro* like they naturally do *in vivo*. Although all these cells sense the lack of gravity within seconds,[20,59] it takes at least 12 h until 3D spheroids are observed[52,53] and up to 7 days until constructs like tubes resembling an intima can be recognized floating in a culture flask.[12,60] These observations are not understood yet by far. Recent research, however, has unveiled a few pieces of the mosaic.

At first, the interaction between extracellular matrix (ECM) and cytoskeletal proteins is affected, when gravity forces are randomized or removed. Most cells studied so far adjust to exposure to microgravity by altering the expression patterns of ECM and cytoskeletal proteins at gene and protein levels.[60–64] Preliminary indications of specific proteins involved in this process came from a comparison of the spheroid formation of the two thyroid cancer cell lines FTC-133 and CGTH-W1. The study revealed that speed of formation and size of resulting spheroids is related to the expression of certain proteins that bind fibronectin.[6] In this context, integrin-α5 was repeatedly determined as important for spheroid formation of human thyroid cells as wells as of porcine hepatocytes.[52,65] Based on ECM–cytoskeleton interaction studies, Ingber postulated that gravity sensation may be due to a change of the forces, which the cells need for their interaction with the environment under the different conditions.[66] This hypothesis is in conformance with recent results that reveal that cells can receive information through integrin-mediated cell–matrix adhesions, which connect the cytoskeleton with the ECM.[67] In this system vinculin plays a decisive role. It is activated when a mechanical stimulus is translated into a biochemical cell response.[68] We found a clear upregulation of vinculin protein in the ML-1 thyroid cancer cells during spheroid formation[10] and Gershovich *et al.* observed an intracellular redistribution of vinculin in mesenchymal stem cells cultured on the RPM.[69] How signals, raised by cell–ECM interaction, are transduced to the cell interior remains to be determined. They may open Ca^{2+} channels allowing an increase of intracellular Ca^{2+} as described by Luo *et al.* for osteoblasts cultured under s-μ*g*.[70] They may be directly transduced to distinct growth factors via the change of the cytoskeleton architecture[71–73] or trigger the phosphorylation of caveolae.[73]

Each system (RPM and RWV) that generates tissue-like cell assembles under microgravity conditions, initially induces an enhanced rate of apoptosis together with an increase in proapoptotic factors.[10,53,60,74–76] In EC, apoptosis seems to impair the angiogenic response to s-μ*g* produced on the RPM.[77] NF-κB1 plays a central role in bone reduction under microgravity conditions.[78] Clejan *et al.* showed that the activation of apoptotic pathways during an early phase of culturing DU-145 human prostate carcinoma cells in a HARV is essential for the subsequent formation of 3D organoids.[79] Our experiments on the RPM on FTC-133 thyroid cancer cells suggested that initiation of an early phase of apoptosis, followed by an escape from the late phase, could be a step in cells that transit from a 2D to a 3D kind of growth.[80] In this process the signaling elements IL-6, IL-8, OPN, TLN1, and CTGF are involved with NF-κB p65. IL-6 and IL-8 together with the von Willebrand factor also play a role in tube formation. After ECs have been cultured on the RPM, precursors of the tubes with single-layered walls could be observed (Fig. 2), in which *IL-8* gene expression was

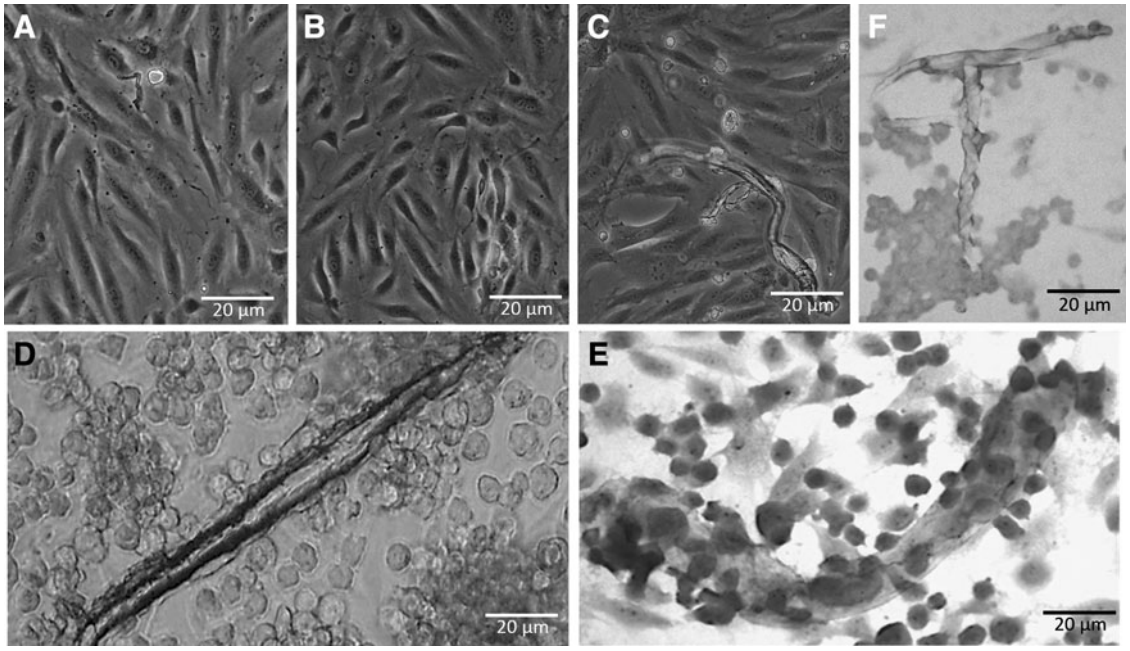

FIG. 2. Tissue engineering of intima constructs. **(A)** Microvascular endothelial cells (MVECs) grown under normal static 1 *g* conditions. **(B)** MVECs grown for 24 h on the RPM. **(C)** MVECs grown for 7 days on the RPM. A tubular construct is visible. **(D)** Coculture of EA.hy926 cells, vascular smooth muscle cells, and fibroblasts for 21 days on the RPM (phase-contrast microscopy). **(E, F)** Sirius Red staining of cocultures of EA.hy926 cells, vascular smooth muscle cells, and fibroblasts, 21 days on the RPM.

Table 2. Proteins or Genes Showing Alterations During Microgravity-Induced Tissue Formation

Cells	*Tissue type*	*Gene*/protein	*Ref.*
ML-1[a]	Spheroids	FAS, BCL-2, vinculin	10
FTC-133[a]	Spheroids	Integrin-α5, myosin-10, filamin B	52
Porcine hepatocytes	Liver morphology	Integrin-α5	65
FTC-133[a]	Spheroids	NF-κB1, interleukin-6	78
FTC-133[a]	Spheroids	*Interleukin-6*, *interleukin-8*, *osteopontin*	80
FTC-133[a]	Spheroids	*EGF*, *CTGF*	11
EA.hy926[b]	Vessels	*Interleukin-8*, *von Willebrand factor*	55
EA.hy926[b]	Vessels	von Willebrand factor, bFGF	60
EA.hy926[b]	Vessels	Matrix metalloproteinase 2	12
HUVEC	–	Caveolin-1	73
Chondrocytes[c]	Cartilage	Collagen type I and X	54

[a]Human thyroid cancer cells.
[b]Human endothelial cells.
[c]Human chondrocytes.
HUVEC, human umbilical vein endothelial cell.

fivefold upregulated and *VWF* gene expression was sixfold downregulated.[55] IL-6, IL-8, and NF-κB p65 are differently regulated by basic fibroblast growth factor depending on whether this growth factor is applied at 1 *g* or s-μ*g*.[16] A brief overview of these observations is given in Table 2. In addition to growth factors, it should also be considered that miRNA expression is influenced by s-μ*g* on the RPM.[81] It is known that several miRNAs have an influence on tube formation *in vitro*.[82,83]

Endothelial Cells

Blood vessel replacements are needed for bypass or reconstructive surgery. Under standard culture conditions, however, ECs grow in a monolayer (Fig. 2A, B) and do not fully exhibit the characteristics they have when they are part of an actual vessel. To obtain a vessel-like structure, endothelial and other cells need to be seeded on preformed scaffolds consisting of biodegradable polymers. While large-caliber constructs generated with this approach usually work well, diameters below 6 mm remain critical due to their increased thrombogenicity.[84–86]

Cultivation of ECs under s-μ*g* might provide a solution for these problems, as ECs are highly sensitive to diverse external stimuli, triggering their activation and modulating their function,[87–91] and strongly react to changes of the influence of gravity. Exposure to r-μ*g* during parabolic flights (22 s) induced dramatic changes in the microtubule network of ECs in the form of tubulin downregulation and rearrangement and altered the expression of 3605 genes.[20,92] Further, gravitational unloading helps the cells to form 3D structures without the need for scaffolds. On the clinostat, the formation of 3D cell aggregates has not been published so far. However, after 24 h of clinorotation, tube formation and migration as well as endothelial nitric oxide synthase (eNOS) activity of human umbilical vein endothelial cells were significantly increased, suggesting an s-μ*g*-mediated promotion of angiogenesis via the PI3K-Akt-eNOS pathway.[93] In addition, Wang *et al.* showed that clinorotation also upregulated inducible NOS (iNOS) in an AP-1-dependent manner and suggested a possible involvement of this pathway in the development of postflight orthostatic intolerance.[94] In parallel to observations in r-μ*g*, the cytoskeleton of ECs grown on the clinostat was remodeled. The expression of various surface adhesion factors was changed, but despite these effects, no critical physiological processes were compromised.[95] Zhang *et al.* reported earlier that mesenchymal stem cells showed an increased endothelial differentiation potential when cultured on a clinostat for 72 h.[96]

Further, clinorotation also has some detrimental consequences for ECs. Kang *et al.* observed that prolonged growth on a clinostat induced among others apoptosis in microvascular ECs.[97]

Moreover, several studies performed on the RWV reported cytoskeletal remodeling, actin downregulation, increased NO release, altered cytokine excretion, and increased caveolin-1 and −2 expression concordantly.[38,72,98–102] Interestingly, reports about the detrimental effects of cultivation on the RWV showed that they depend on the origin of the investigated cells. Mariotti and Maier reported that only microvascular cells suffer from inhibited growth and angiogenesis, while macrovascular cells proliferated faster when grown on an RWV.[103]

Exposing the cells to altered gravity conditions on an RPM led an increased apoptosis rate,[17,53] which was significantly reduced by the addition of vascular endothelial growth factor to the culture medium.[104] Similar effects have also been reported in further studies.[105] More importantly, Ea.hy926 cells readily formed 3D multicellular spheroids similar to those observed on RWVs within the first 24 h of incubation on the RPM.[53,104] Between the 5th and the 7th day of growth on the RPM, double-row cell assemblies were forming. In the course of the next weeks, these assemblies developed into tube-like structures with walls mostly consisting of single-cell layers. They were found to have a lumen and resembled the intima of blood vessels *in vivo*, reaching a length of up to several centimeters.[12,60] These structures are unlike any other constructs generated by s-μ*g* tissue engineering either on clinostats or RWVs and are presently the most promising candidates on the way to the engineering of a complete, functional vessel (Fig. 2C–F). First investigations in both short- and long-term s- and r-μ*g* have shown that this process involves a complex interplay of a multitude of molecules belonging to the cytoskeleton,

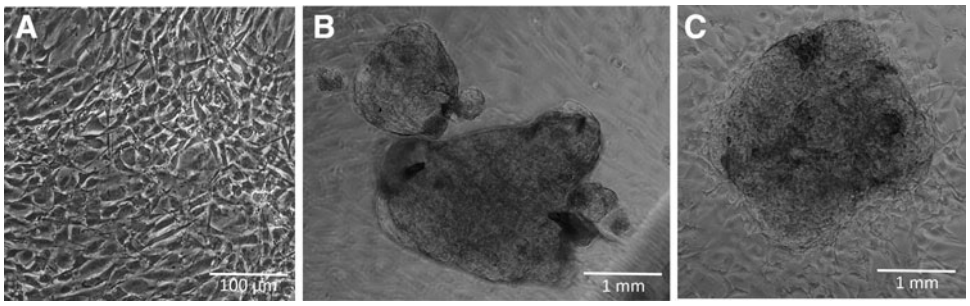

FIG. 3. Cartilage formation under simulated microgravity. **(A)** Control sample after 14 days of cultivation under normal gravity conditions. **(B)** Three-dimensional aggregates of human chondrocytes after 14 days of cultivation on the RPM. **(C)** Three-dimensional aggregate grown for 21 days in an RWV and seeded out on a slide flask. Cells are growing out from the spheroid.

ECM, apoptotic processes, cytokines, angiogenesis, and cell cycle with a total number of over 1600 genes identified as differentially regulated on the RPM.[20,55,92,105]

Cartilage

Interest in tissue engineering of cartilage is motivated by the inability of adult articular cartilage to repair itself after damage by disease or injury.[106,107] Chondrocytes are the most obvious cell source for the tissue engineering of cartilage.[108] However, chondrocyte expansion in a monolayer causes cell dedifferentiation, characterized by decreased proteoglycan synthesis and type II collagen expression, and increased type I collagen expression.[109–111]

Cartilage development is achieved *in vitro* by cultivating living cells on biodegradable polymer scaffolds.[112] The scaffold should (1) be biodegradable in a controlled way without cytotoxic, tumorigenetic, nephrotoxic, or other undesirable effects; (2) have a porosity that allows diffusion of nutrients and waste products; (3) support cell viability, proliferation, differentiation, and ECM production; (4) be able to fix to and integrate with the tissue at the defect site; and (5) give mechanical support to the engineered tissue.[104,106] Ideally, as the ECM develops, the scaffold dissolves and disappears.[107]

A comparison of chondrocyte cells cultured in rotating bioreactors in Space (Mir Space Station) and on Earth was reported by Freed *et al.*[113] Freed and his team performed experiments with rotating cultures of bovine chondrocytes in polyglycolic acid scaffolds and concluded that after 7 months of cultivating the culture on Earth (static 1 *g* control) it produced cartilage tissues closer to the natural form than that in Space. Very recently, the scaffold-free chondrogenesis of human cartilage in an RWV bioreactor has been reported.[114] Good quality cartilage tissue was formed by a rotating culture from aged human articular cartilage after 90 days of cultivation. The report of Ohyabu *et al.*[115] was the first on the production of cartilaginous tissue from bone-marrow-derived cells by a rotating culture. A comparison of porcine chondrocyte cells cultured on the International Space Station (ISS) and on the RPM was firstly done by Stamenković *et al.*[116] Neocartilage formed in normal gravity was ''continuous in shape and form that corresponded to the shape of the cylindrical bioreactor,''[116] compared to ISS and RPM tissues, which consisted of irregular cell aggregates.[116] RPM-cultivated chondrocytes created tissue with matrix composition characteristics in between the control 1 *g* and the ISS-produced neocartilages, but produced neocartilage with more evenly dispersed and further spaced apart cells.[54] The number of cells that can be obtained from a clinically relevant-sized biopsy is not sufficient for cell-based therapies such as autologous cartilage implantation or to produce a functional neocartilage implant. Thus, the classic expansion phase in monolayer is needed to increase cell numbers, adding to the probability of reduced chondrogenic capacities.[116] This point is especially a factor in scaffold-free methods that rely on the volume of cells to act as their own scaffold support and tissue volume.[114] The RPM therefore offers both opportunities: a reduction in the amount of cells and the prefabrication of implants without the complications associated with scaffolds (Fig. 3). Thus, the RPM has proved to be suitable for tissue engineering of cartilage.[34]

Thyroid

The first observations of altered thyroid function, that is, thyroid hormones, in astronauts were already made in the late 1970s[117] and could be confirmed in later studies on humans as well as mammals.[117–119] Since the beginning of the new millennium, three Space missions were carried out that either analyzed thyroid rat cells[120] or thyroids of mice[121] flown in Space. Based on the ratio of sphingomyelinase and sphingomyelin synthase, two enzymes responsible for the sphingomyelin metabolism, Albi *et al.* were able to determine the state of rat thyroid cells (FRTL-5) returning from r-µ*g* as proapoptotic.[120] Similar changes of the sphingomyelin metabolism could be detected in a single mouse returning after 91 days to Earth. Further, it could be shown that beside the activity of the proteins their location in the thyroid cells was altered.[121] Overall, obvious differences in the thyroid tissue structure could be detected.[122,123]

Investigating the effects generated by an RPM, changes in the cytoskeleton, the ECM, and in apoptosis of human thyroid cells were observed.[10,124,125] By proteome analyses, normal and cancerous thyroid-cell-dependent changes in the content of regulatory, metabolic, and structural proteins could be detected.[52,126,127] The proteome analyses were carried out with samples of human thyroid cells cultured on an RPM for several days, during which they formed small spherical aggregates (spheroids, Fig. 4). Attempts to identify

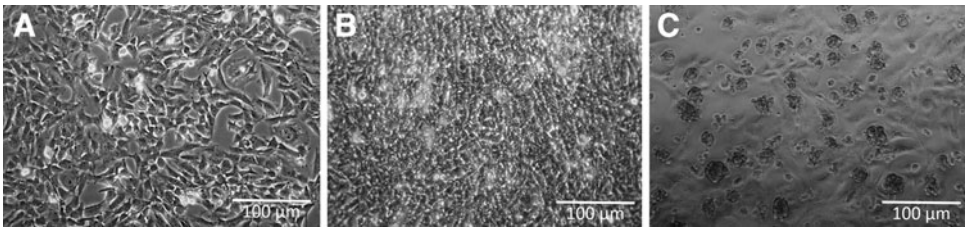

FIG. 4. Formation of multicellular tumor spheroids. **(A)** Static control at start of the experiment with the poorly differentiated follicular thyroid carcinoma cell line CGTH-W1. **(B)** Static control at day 4 at the end of the experiment, CGTH-W1 cell line. **(C)** RPM experiment, 4 days, adherent cells were visible as well as multicellular tumor spheroids (CGTH-W1 cell line) floating in the supernatant.

proteins that may be involved in spheroid formation hinted to various isoforms of the adhesion molecule integrin.[52,128] A change of the molecule, however, could not be detected on the RNA level in human thyroid cells cultivated for 14 days in an RWV and thus was deemed not responsible for spheroid formation.[129] It may be that the integrin protein is essential in the early stages of spheroid formation but later on other proteins are responsible for sustaining the spheroids. Parabolic flights revealed that the cytoskeleton of thyroid carcinoma cells begins to change already after 22 s of exposure to r-µ*g* for 22 s.[59]

Summary

The preferred environment for tissue engineering studies would be a long-term flight in the microgravity environment, but devices, such as clinostats, the RPM, or RVW, are very useful tools in this area.

Recent research revealed that the formation of 3D aggregates under s-µ*g* can be divided into three phases of regulation that cells pass through when they transit from a 2D to a 3D kind of growth under gravitational unloading: (1) cells sense a change of the forces required for interaction with the ECM, (2) they face an initiation of apoptosis, and (3) they transduce the signals generated during phases 1 and 2 to the nucleus via kinase-dependent pathways.

Growing cells under r- or s-µ*g*, particularly on the RPM, became a very promising approach to generate tissue constructs in a scaffold-free manner. Using human ECs to form intima constructs might be a first step to the generation of fully functional vessels usable in both *in vitro* research and (reconstructive) surgery.

In addition, thyroid cells, which can also be stimulated by s-µ*g* to grow in form of 3D aggregates, can in future be grown up to a state to resemble thyroid tissue. Currently, studies that analyze the molecular mechanisms behind spheroid formation have increased the knowledge of the complex regulation of 3D growth in microgravity.[52,55,80,130–132]

Further, cartilage may be formed on the RPM and RVW. The advantage of this procedure is that cell aggregation and cell proliferation are simultaneously going on so that the number of cells required at the beginning can be kept low even if an enlarged piece of cartilage is desired. However, the biomechanical stability of 3D chondrocyte aggregates produced under conditions of s-µ*g* has still to be tested.

Taken together, the use of microgravity to study human cells is very important to understand the exact cellular changes occurring under these special conditions. Hence, Space medicine has an important contribution to the progress in basic research. The tissues, engineered from single cell layers cultured in s-µ*g*, might be used in the future for medical transplantations. Tissue-engineered cartilage may be important for regenerative medicine. It is also important that with the help of these 3D structures, which are similar to the *in vivo* situation, studies on the molecular mechanisms of processes such as angiogenesis or tumor cell apoptosis are possible without animal testing.

Experiments conducted in Space or in s-µ*g* environment are not typically the studies a molecular or cellular biologist is able to perform. We have learnt from Space research that the compensation of gravity impacts on the cells enormously and dramatically changes the genome, proteome, and secretome of these cells. However, a spaceflight and a stay on the ISS provides physical conditions that are not achievable on Earth, but can be exploited to study mechanisms and pathways that control cell growth and function. Over the last 40 years, many experiments have shown that exposure to microgravity alters biological processes that may be relevant to the growth behavior and function of human cells. Several spaceflights have demonstrated 3D growth in Space and similar results were detected with the help of devices simulating microgravity in our laboratories.[11,113,116,133] Therefore, this promising research area can help to develop new strategies for tissue engineering.

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