Chapter 6
Tissue Engineering in Microgravity

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Abstract Tissue engineering enables the development of functional constructs from cells and has different applications in regenerative medicine and drug screening but also in non-therapeutic approaches.

In the course of several space flight missions as well as ground-based experiments, it has been shown that both real and simulated microgravity can induce the formation of three-dimensional tissues in different human cell types. Apart from scaffold-based approaches, which are also employed under normal gravity conditions on Earth, microgravity offers unique conditions to facilitate a scaffold-free development of three-dimensional multicellular aggregates or spheroids and even organotypic tissue. So far, the underlying mechanisms of the observed spontaneous cell aggregation are not yet known, but they are subject to intensive investigation in the gravitational biology community. This knowledge can contribute to an optimization of three-dimensional tissue growth on different microgravity platforms and to the understanding of scaffold-free tissue engineering. Additionally, these constructs provide an efficient tool for downstream experiments such as drug testing and could be used as a replacement for *in vivo* models, thereby reducing the need for animal testing. Furthermore, future applications such as medical transplants are possible. This chapter will present an overview of the current state of microgravity-based tissue engineering.

Keywords Microgravity • Tissue Engineering • Spheroids • Cartilage • Bone • Endothelium

6.1 Introduction

Tissue engineering, a term first coined at the National Science Foundation Forum on Issues, Expectations, and Prospects for Emerging Technology Initiation, held at Granlibakken Resort, Lake Tahoe, California, in February 1988 and later refined by Robert Langer and Joseph P. Vacanti, is defined as “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of

G. Ruyters et al., *Biotechnology in Space*, SpringerBriefs in Space Life Sciences, DOI 10.1007/978-3-319-64054-9_6

biological substitutes that restore, maintain, or improve tissue function" employing the use of isolated cells or cell substitutes, tissue-inducing substances or cells placed on or within matrices (Langer and Vacanti 1993).

Early experiments under real microgravity (r-µ*g*) in space on board different Space Shuttle missions revealed that weightlessness has an influence on the aggregation behavior of human cells. Tschopp et al. found that suspended human embryonic kidney cells tended to attach to carrier microbeads (Tschopp et al. 1984), while Dintenfass observed an aggregation of red blood cells in space (Dintenfass 1986). These results indicated that microgravity might be beneficial for the formation of three-dimensional cell aggregates and led to further studies, investigating this phenomenon more thoroughly.

However, the increasing interest in the application of microgravity and the low availability of actual space flight opportunities meant that studying µ*g* on Earth soon also came into focus. Unfortunately, because of the very short µ*g* exposure time during parabolic flights (22 s) or sounding rocket missions (6 min), these two options are only of limited use for tissue engineering purposes. Therefore, devices for the simulation of microgravity (s-µ*g*) have also been employed since the very early stages of µ*g*-assisted tissue engineering. Most prominently, the NASA-developed Rotating Wall Vessel (RWV) bioreactor (Klaus 2001; Schwarz et al. 1992; Hammond and Hammond 2001) has been used for cells (with or without scaffolds) in suspension, while the Random Positioning Machine (RPM) (Borst and van Loon 2008; van Loon 2007) or the fast-rotating clinostat (FRC) (Eiermann et al. 2013) were preferred for adherent cell cultures. All these machines keep the samples in constant motion/rotation. The RWV counteracts the gravitational vector by rotating the circular culture vessel around a horizontal axis at a speed where the upward fluid flow of the medium and the downward sedimentation of the cells are a balance. This keeps the cells in a state of constant free fall. The FRC and the RPM rotate the culture flasks around one or all three axes in space leading to a mean annulled influence of the gravitational vector over time.

Under normal gravity conditions, isolated cells cultured in regular culture flasks will only grow in a monolayer (2D). In order to produce three-dimensional tissue constructs, it is therefore often necessary to introduce a so-called "scaffold", a structure that provides a surface for the cells to attach to, determines the shape and contributes to the overall mechanical stability of the generated tissue. Scaffolds are usually made from materials such as hydroxyapatite (HA), D,L-polylactic-polyglycolic acid (PLGA), bioactive glass, L-polylactic acid (L-PLA), polycaprolactone (PCL) or poly(ethylene glycol)-terephthalate (PEG/PBT) (Hollister 2005; Dutta et al. 2017). However, while helping the cells to assemble in a 3D structure in the initial phase of the tissue engineering process, the scaffolds might eventually pose some problems in the long run, such as unforeseen immunologic problems, a distorted structure of the newly formed tissue or an altered mechanical resilience compared to natural tissues. Therefore, the ultimate aim of tissue engineering is the *de novo* formation of scaffold-free, functional, organotypic tissue constructs. Employing microgravity-based tissue engineering techniques might be a step further in this direction (Grimm et al. 2014).

6.2 Tissue Engineering in Simulated Microgravity

A wide spectrum of different cell and tissue types has been used for tissue engineering studies using s-μ*g* on devices such as the RWV or the RPM. Compared to experiments in space, they have the advantages of a higher number of replicates, better control of the environment (temperature, humidity, atmospheric CO_2 concentration), a higher throughput of samples, easier accessibility of suitable facilities and highly reduced costs. On the other hand, it has to be considered that both machines can only approximate r-μ*g* conditions to a certain extent, as residual acceleration, shear forces and disturbances by bubbles are inherent to their functional principle (Wuest et al. 2015; Hammond and Hammond 2001; Lappa 2003). Nevertheless, s-μ*g*-based techniques have been the methods of choice for the majority of tissue engineering approaches.

6.2.1 Cartilage

In 1991, the first report of cartilage tissue engineering in s-μ*g* showed that rat embryonic limb mesenchymal cells growing on microcarrier beads in a RWV bioreactor eventually differentiated into functional chondrocytes, producing Alcian-blue positive matrix. Furthermore, over the 65-day experiment duration cells and microcarriers aggregated and the newly formed 3D structures kept increasing in size (Daane et al. 1991; Duke et al. 1993).

Similar observations were made in several following experiments, where in a RWV, chondrocytes of different origins seeded on polymer scaffolds formed macroscopically large (with lengths of each edge in the range of several mm) three-dimensional aggregates. The resulting tissues were very similar to natural cartilage, exhibiting comparable cell densities, glycosaminoglycan (GAG) and collagen II percentages. Furthermore, tissue constructs deriving from s-μ*g* conditions were mechanically and structurally superior to those generated in spinner flasks or in Petri dishes (Baker and Goodwin 1997; Freed et al. 1998; Freed and Vunjak-Novakovic 1997; Falsafi and Koch 2000; Gorti et al. 2003; Wu et al. 2013). It could also be shown that TGF-β_1 supplementation of the growth medium (5 ng/mL) resulted in an improved proteoglycan production of rat articular chondrocytes cultured on three-dimensional macroporous PLGA sponges for 4 weeks in a RWV (Emin et al. 2008).

The first scaffold-free generation of cartilage tissue in s-μ*g* was reported by Conza et al. (2001). As a preparation for a space flight experiment, chondrocytes were seeded into a specially designed hardware intended for use on the ISS and were cultured on an RPM for up to 3 weeks. The culture chamber geometry was cylindrical with a diameter of 8 mm and a height of either 8 or 2 mm. Cartilage tissue constructs obtained from the RPM were round in shape, in contrast to those from static controls, whose shape followed that of the culture chamber. The chondrocytes also exhibited a more ordered arrangement than those grown in 1*g*. Later results, however, showed that cartilage grown in the same hardware on the ISS was inferior to the material from the ground controls and that the RPM samples had an intermediate quality (Stamenkovic et al. 2010).

Scaffold-free engineering of cartilage tissue has also been demonstrated using dedifferentiated chondrocytes in an RWV bioreactor. After 90 days of culture, a dense collagen-II- and proteoglycan-rich cartilaginous tissue was found consisting of highly metabolically active chondrocytes (Marlovits et al. 2003).

Another scaffold-free approach was used by Aleshcheva et al. (Aleshcheva et al. 2016, Grimm et al. 2014). Adherent chondrocytes were cultured for up to 21 days on an RPM. At the end of this period, some chondrocytes had spontaneously detached from the bottom of the culture flasks and formed multicellular spheroids suspended in the tissue culture medium. Their size was also in the mm range, but overall smaller in comparison to their scaffold-supported counterparts. First studies to elucidate the possible mechanisms of this scaffold-free cartilage growth employing parabolic flights and further experiments on the RPM indicated that genes involved in the mechanical properties of the cells as well as adhesion, growth and apoptosis were regulated upon exposure to μ*g*. Furthermore, it could be shown that during cultivation on the RPM the chondrocytes switched from collagen I and –X production towards collagen II, chondroitin sulphate and aggrecan production (Ulbrich et al. 2010; Aleshcheva et al. 2013, 2016).

Besides employing already differentiated chondrocytes, it was also demonstrated by several groups that mesenchymal stem cells (MSCs) could be induced to differentiate into a chondrocyte phenotype in RWV bioreactors. A scaffold-free method has been described by Ohyabu et al. (2006), generating large ($1.25 \pm 0.06 \times 0.60 \pm 0.08$ cm) cartilaginous tissue constructs from suspended rabbit bone marrow cells cultivated in an RWV for 3 weeks. Collagen I, II, safranin-O and toluidine blue staining together with the gene expression patterns of aggrecan, and collagens I and II as well as the glycosaminoglycan/DNA ratio confirmed the cartilaginous properties of the tissue. The possible role of TGF-β_1 is still debated, as one study showed no influence of this molecule on the s-μ*g*-induced differentiation of MSCs into chondrocytes (Luo et al. 2011), while other authors showed that s-μ*g* and TGF-β_1 synergistically promote the differentiation into chondrocytes by activating the p38 MAPK pathway (Yu et al. 2011). However, very recently, it was reported that mesenchymal stem cells differentiated into chondrocytes without the use of an exogenous growth factor when cultivated on decellularized cartilage ECM-derived particles in a RWV for 21 days. The resulting cartilage microtissue aggregates. Most interestingly, these constructs, when implanted with fibrin glue into a rat model for cartilage defects, were shown to improve and accelerate joint function recovery and cartilage repair in comparison to the microtissue constructs or fibrin glue alone.

6.2.2 Thyroid Cancer Spheroids

S-μ*g* has been identified as a means to produce spheroids from different types of malignant cells early on. Multicellular tumor spheroids (MCTSs) offer many possibilities for further studies of tumor development, metastasis, host-tumor interactions and drug testing, among others (Jessup et al. 1993; Ingram et al. 1997).

Currently, the majority of spheroids used for these kinds of analyses are still generated under classical 1*g*conditions, as illustrated by a selection of the most recent publications (Halfter et al. 2016; Akasov et al. 2016; Ravi et al. 2016; Wang et al. 2016). More in-depth reviews are given in Mehta et al. (2012) and Wang et al. (2014). However, s-µg-generated spheroids might be superior to their 1g counterparts, as culture conditions allow for a more physiological structure of the tissue constructs, undisturbed by any potentially interfering sedimentation force, thereby simplifying the translation from *in vitro* results to *in vivo* applications. Due to the diversity of different MCTSs generated under s-µ*g*, this paragraph will focus on thyroid cancer cells.

Using the RPM, Grimm et al. were successful in generating MCTSs from the adherent thyroid carcinoma cell lines ML-1 and FTC-133 (Grimm et al. 2002; Pietsch et al. 2011). It was found that s-µ*g* induced increased apoptosis in both cell lines, possibly reflecting the reduction of thyroid function observed in astronauts (Strollo 1999). Both proteomic and genomic analyses of FTC-133 MCTSs vs. 1g control cultures revealed that during spheroid formation the cells express fibronectin-binding surface proteins, thereby strengthening the cell-to cell adhesion (Pietsch et al. 2011), and that the genes *IL-6*, *IL-8*, *OPN*, *TLN1*, *CTGF*, *NGAL*, *VEGFA*, *IL17*, *VEGFD*, *MSN*, *MMP3*, *ACTB*, *ACTA2*, *KRT8*, *TUBB*, *EZR*, *RDX*, *MSN*, *PRKCA*, *MMP9*, *PAI1* and *MCP1* were generally regulated in such a manner that they upregulated genes coding for proteins, which promote 3D growth (angiogenesis) and prevent excessive accumulation of extracellular proteins, while gene coding for structural proteins is downregulated in MCTSs (Pietsch et al. 2011; Grosse et al. 2012; Warnke et al. 2014; Kopp et al. 2015; Riwaldt et al. 2015a, 2016).

6.2.3 Bone

Bone tissue is one of the most researched aspects in the field of tissue engineering in µ*g*. So far, however, all efforts have been confined to experiments in s-µ*g*.

The first step in bone tissue engineering was reported in 1998 by Qiu et al. (1998). Secondary rat marrow stromal cells were cultured for 2 weeks on Cytodex-3 microcarrier beads in an RWV and formed spherical aggregates exhibiting mineralization as well as alkaline phosphatase activity and collagen type I and osteopontin expression. Over the years, the technique for bone tissue engineering was further refined, but in principle, it is always a variation of using either osteoblasts or mesenchymal stem cells grown on different scaffold (interconnected porous HA, porous PLGA, bioactive glass-polymer composites, human bioderived bone scaffolds, alginate or gelatin) cultures in an s-µ*g* device, usually an RWV. Most studies showed that the s-µ*g*-derived tissue was comparable to natural bone and usually superior to engineered tissue from static cultures, as evidenced by their greater *in vivo* effectiveness in repairing bone lesions in animal models (Sikavitsas et al. 2002; Nishikawa et al. 2005; Song et al. 2006, 2007, 2008; Hwang et al. 2009; Lv et al. 2009; Jin et al. 2010; Cerwinka et al. 2012; Ulbrich et al. 2014).

6.2.4 *Endothelium*

Endothelial cells, the inner lining of the blood vessels, play an important role in many physiological processes in the human body, most notably in the regulation of blood pressure. Lesions in the endothelium can lead to life-threatening complications, such as infarctions. Therefore, endothelial repair/blood vessel replacement is an important topic in modern medicine. Furthermore, for complicated (micro) surgical procedures it could be advantageous to produce autologous vessels to circumvent possible rejections of important grafts.

Three-dimensional endothelial cell constructs in a RWV were first generated by Sanford et al. (2002). Bovine aortic endothelial cells were first seeded onto Cytodex-3 microcarrier beads and then cultivated in s-µ*g* for 30 days. The authors found large tissue-like aggregates consisting of at least 20 beads and viable cells of typical endothelial cell morphology, forming multilayered sheet-like structures separated by a zone of matrix material. The cells showed tenfold enhanced NO production compared to Spinner flask control cultures, which was inducible by l-arginine and blockable by L-NAME, indicating a physiological behavior. Furthermore, they showed increased barrier properties.

In 2005, CD34+ human umbilical cord stem cells were cultured in s-µ*g* using RWVs with or without Cytodex-3 microcarrier beads for 14 days. The growth medium contained 50 ng/mL vascular endothelial growth factor (VEGF). Interestingly, on day 4 the cells cultured in the absence of microcarrier beads formed three-dimensional aggregates resembling tubular structures, whereas in the bead-containing RWVs only amorphic cell clusters were found. FACS analyses revealed that the cells in the tubular structures expressed endothelial markers such as CD34, CD31 and flk1 and microscopically they exhibited the morphologies of vascular endothelial-like cells and spindle cells (Chiu et al. 2005). In accordance with this study, it was later confirmed that s-µ*g* conditions in a clinostat lead to differentiation of mesenchymal stem cells into an endothelial phenotype, expressing typical endothelial markers such as Flk-1 and vWF (Zhang et al. 2013).

Using the RPM to culture the immortalized endothelial cell line EA.hy926, a fusion of human umbilical vein endothelial cells (HUVECs) with a thioguanine-resistant clone of A549 adenocarcinoma cells (Edgell et al. 1990), with and without a supplementation of 10 ng/mL VEGF in the growth medium for 72 h, Infanger et al. (2006) observed an initial increase in the expression of extracellular matrix proteins induced by both s-µg and VEGF alone, which was further augmented by s-µ*g* after 12 h. In addition, s-µ*g* induced apoptosis beginning from four h culture time and increasing until 72 h, while VEGF reduced the apoptosis rate. After 72 h, the authors also found that many non-apoptotic cells had formed tube-like aggregates. These tubes were further characterized and it was found that adherent Ea. hy926 cultured on an RPM began to form small colonies by spreading over neighboring cells. From these colonies, tube-like structures emerged after 2 weeks of

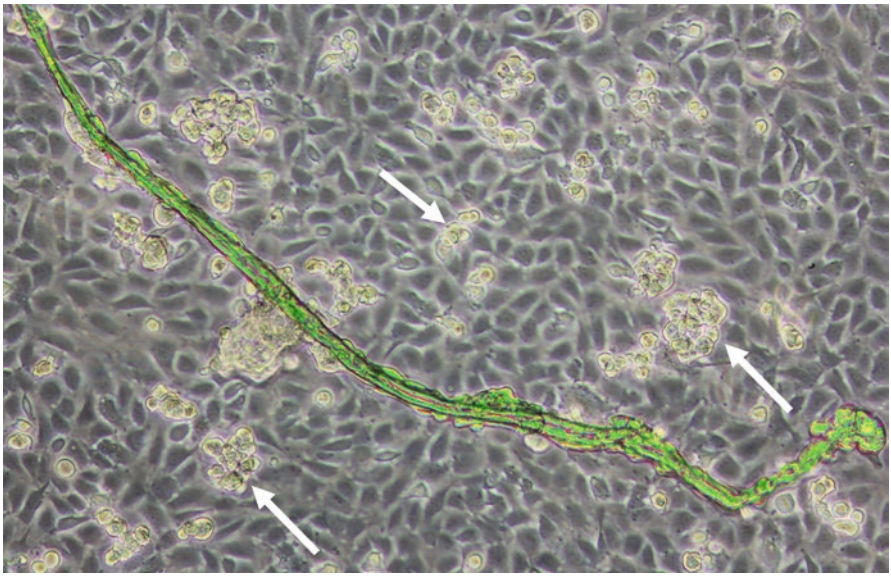

Fig. 6.1 Endothelial tubular structure after 21 days culturing on the RPM. All three types of cell growth observed on the RPM are shown. In the background a 2D monolayer of adherently, growing endothelial cells can be seen, while two kinds of 3D aggregates are also present. *White arrows* point out multicellular spheroids, a large tubular structure similar to an intima, is highlighted in *green* for better visibility

cultivation, which formed a defined lumen and continued to elongate over the course of 2 more weeks of RPM culture. The tube walls resembled vascular intimas and consisted of a single layer of cells (Fig. 6.1), which produced more β1-integrin, laminin, fibronectin and α-tubulin than 1*g* control cells.

It can therefore be assumed that the specific s-μ*g* culture conditions on an RPM offer a unique opportunity to study the mechanisms of 3D vessel development (Grimm et al. 2009). The first studies to elucidate the mechanism of tube formation hinted at an involvement of phosphokinase cα and of an interaction network formed by the genes *RDX*, *EZR*, *MSN*, *GSN*, *CALD1*, *SPTAN1*, *VIM*, *TLN1*, *ITGB1*, *CAV1*, *ANXA2*, *ICAM1*, *ENG*, *SERPINE1*, *IL6* and *IL8* (Grimm et al. 2010; Ma et al. 2013).

6.3 Tissue Engineering in Real Microgravity

Compared to tissue engineering approaches on Earth in an s-μ*g* environment, conducting such projects in space is a far more technically, logistically and, of course financially challenging endeavor. Therefore, their absolute number is relatively small.

6.3.1 Cartilage

The first attempts to grow cartilage tissue constructs during space missions were undertaken by Freed et al. (Freed et al. 1997; Saltzman 1997). This was a long-term experiment lasting a total of 7 months. The authors first generated three-dimensional cell-polymer constructs from bovine articular chondrocytes and polyglycolic acid scaffolds in rotating bioreactors on Earth over a period of 3 months. Afterwards, one reactor containing ten 3D constructs was transported to the *MIR* space station and the cultivation continued under r-µ*g* for a further 4 months. In parallel, a second bioreactor with ten constructs was left on Earth in 1*g* and was operated for the same time. Under both gravitational conditions, functional and viable cartilaginous constructs emerged. However, the r-µ*g* samples tended to possess an overall rounder shape, smaller size and reduced mechanical stability when compared to those grown on Earth (Freed and Vunjak-Novakovic 1997; Freed et al. 1999).

A scaffold-free approach was used for the generation of neocartilage derived from porcine chondrocytes. The cells were seeded in cylindrical culture chambers and subsequently exposed to r-µ*g* on board the ISS, s-µ*g* on an RPM and, as a control, 1g in a stationary set-up on Earth (Stamenkovic et al. 2010; Conza et al. 2001). The experiment lasted for 16 days, after which the tissue was subjected to histological and gene expression analyses. The authors found that, compared to those from s-µ*g* and 1*g*, the samples from the ISS showed a weaker stain for extracellular matrix. The ISS samples also possessed a higher collagen II/I expression ratio than control tissue. On the other hand, aggrecan/versican expression and cell density were increased in 1*g* tissues compared to both r- and s-µ*g*. These results are in accordance with those found by Freed et al. (1997) and seem to reflect the observed average loss of about 8% of cartilage thickness after 14 days of mechanical unloading during a 6-degree head-down-tilt bedrest in young healthy subjects (Liphardt et al. 2009).

6.3.2 Thyroid Cancer Spheroids

Due to its tolerance to culture temperatures well below the ideal 37 °C, the human follicular thyroid cancer cell line FTC-133 was chosen for two space flight missions, aimed at generating MCTSs under r-µ*g*. The first mission, SIMBOX on Shenzhou-8 in 2011, was conducted for 10 days, using a specially designed cell culture hardware by Airbus Defence and Space. After the flight, several MCTSs were found inside the culture vessels, which were considerably bigger (about 4–5 mm in diameter) than comparable MCTSs generated on an RPM in a parallel control experiment (Pietsch et al. 2013). Subsequent analyses of the MCTSs and the cell culture supernatants suggested that *EGF* and *CTGF* might be involved in r-µ*g*-induced MCTS formation and that a regulation of *IL6*, *IL8*, *IL15*, *OPN*, *VEGFA*, *VEGFD*, *FGF17*, *MMP2*, *MMP3*, *TIMP1*, *PRKAA* and *PRKACA* in r-µ*g* (and s-µ*g*

RPM control experiments) might shift the cells towards a less aggressive phenotype (Pietsch et al. 2013; Ma et al. 2014).

The second space-flown experiment was CellBox-1 in 2014, essentially designed as a replicate of the SIMBOX experiment, this time conducted for 10 days in the ESA Columbus module of the ISS. However, due to launch delays, the protocol for cell culture had to be adapted to the new situation. This led to an overgrowth of cells on the ground, ultimately preventing the formation of MCTSs in space. However, this led to the finding that an increased production of extracellular matrix-related proteins has the potential to prevent spheroid formation in r-µ*g* (Riwaldt et al. 2015b).

References

Akasov R, Zaytseva-Zotova D, Burov S, Leko M, Dontenwill M, Chiper M, Vandamme T, Markvicheva E (2016) Formation of multicellular tumor spheroids induced by cyclic RGD-peptides and use for anticancer drug testing *in vitro*. Int J Pharm 506(1–2):148–157. doi:10.1016/j.ijpharm.2016.04.005

Aleshcheva G, Sahana J, Ma X, Hauslage J, Hemmersbach R, Egli M, Infanger M, Bauer J, Grimm D (2013) Changes in morphology, gene expression and protein content in chondrocytes cultured on a random positioning machine. PLoS One 8(11):e79057. doi:10.1371/journal.pone.0079057

Aleshcheva G, Bauer J, Hemmersbach R, Slumstrup L, Wehland M, Infanger M, Grimm D (2016) Scaffold-free tissue formation under real and simulated microgravity conditions. Basic Clin Pharmacol Toxicol 119(suppl 3):26–33. doi:10.1111/bcpt.12561

Baker TL, Goodwin TJ (1997) Three-dimensional culture of bovine chondrocytes in rotating-wall vessels. In Vitro Cell Dev Biol Anim 33(5):358–365. doi:10.1007/s11626-997-0006-5

Borst AG, van Loon JJWA (2008) Technology and developments for the random positioning machine, RPM. Microgravity Sci Technol 21(4):287. doi:10.1007/s12217-008-9043-2

Cerwinka WH, Sharp SM, Boyan BD, Zhau HE, Chung LW, Yates C (2012) Differentiation of human mesenchymal stem cell spheroids under microgravity conditions. Cell Regen (Lond) 1(1):2. doi:10.1186/2045-9769-1-2

Chiu B, Wan JZ, Abley D, Akabutu J (2005) Induction of vascular endothelial phenotype and cellular proliferation from human cord blood stem cells cultured in simulated microgravity. Acta Astronaut 56(9–12):918–922

Conza N, Mainil-Varlet P, Rieser F, Kraemer J, Bittmann P, Huijser R, van den Bergh L, Cogoli A (2001) Tissue engineering in space. J Gravit Physiol 8(1):17–20

Daane E, Duke PJ, Campbell M (1991) Chondrogenesis of limb mesenchymal cells cultured on microcarrier beads. TSEMJ 22(1):52

Dintenfass L (1986) Execution of "ARC" experiment on space shuttle "Discovery" STS 51-C: some results on aggregation of red blood cells under zero gravity. Biorheology 23(4):331–347

Duke PJ, Daane EL, Montufar-Solis D (1993) Studies of chondrogenesis in rotating systems. J Cell Biochem 51(3):274–282. doi:10.1002/jcb.240510306

Dutta RC, Dey M, Dutta AK, Basu B (2017) Competent processing techniques for scaffolds in tissue engineering. Biotechnol Adv 35(2):240–250. doi:10.1016/j.biotechadv.2017.01.001

Edgell CJ, Haizlip JE, Bagnell CR, Packenham JP, Harrison P, Wilbourn B, Madden VJ (1990) Endothelium specific Weibel-Palade bodies in a continuous human cell line, EA.hy926. In Vitro Cell Dev Biol 26(12):1167–1172

Eiermann P, Kopp S, Hauslage J, Hemmersbach R, Gerzer R, Ivanova K (2013) Adaptation of a 2-D clinostat for simulated microgravity experiments with adherent cells. Microgravity Sci Technol 25(3):153–159. doi:10.1007/s12217-013-9341-1

Emin N, Koc A, Durkut S, Elcin AE, Elcin YM (2008) Engineering of rat articular cartilage on porous sponges: effects of tgf-beta 1 and microgravity bioreactor culture. Artif Cells Blood Substit Immobil Biotechnol 36(2):123–137. doi:10.1080/10731190801932116

Falsafi S, Koch RJ (2000) Growth of tissue-engineered human nasoseptal cartilage in simulated microgravity. Arch Otolaryngol Head Neck Surg 126(6):759–765

Freed LE, Vunjak-Novakovic G (1997) Microgravity tissue engineering. In Vitro Cell Dev Biol Anim 33(5):381–385. doi:10.1007/s11626-997-0009-2

Freed LE, Langer R, Martin I, Pellis NR, Vunjak-Novakovic G (1997) Tissue engineering of cartilage in space. Proc Natl Acad Sci U S A 94(25):13885–13890

Freed LE, Hollander AP, Martin I, Barry JR, Langer R, Vunjak-Novakovic G (1998) Chondrogenesis in a cell-polymer-bioreactor system. Exp Cell Res 240(1):58–65. doi:10.1006/excr.1998.4010

Freed LE, Pellis N, Searby N, de Luis J, Preda C, Bordonaro J, Vunjak-Novakovic G (1999) Microgravity cultivation of cells and tissues. Gravit Space Biol Bull 12(2):57–66

Gorti GK, Lo J, Falsafi S, Kosek J, Quan SY, Khuu DT, Koch RJ (2003) Cartilage tissue engineering using cryogenic chondrocytes. Arch Otolaryngol Head Neck Surg 129(8):889–893. doi:10.1001/archotol.129.8.889

Grimm D, Bauer J, Kossmehl P, Shakibaei M, Schoberger J, Pickenhahn H, Schulze-Tanzil G, Vetter R, Eilles C, Paul M, Cogoli A (2002) Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. FASEB J 16(6):604–606

Grimm D, Infanger M, Westphal K, Ulbrich C, Pietsch J, Kossmehl P, Vadrucci S, Baatout S, Flick B, Paul M, Bauer J (2009) A delayed type of three-dimensional growth of human endothelial cells under simulated weightlessness. Tissue Eng Part A 15(8):2267–2275. doi:10.1089/ten.tea.2008.0576

Grimm D, Bauer J, Ulbrich C, Westphal K, Wehland M, Infanger M, Aleshcheva G, Pietsch J, Ghardi M, Beck M, El-Saghire H, de Saint-Georges L, Baatout S (2010) Different responsiveness of endothelial cells to vascular endothelial growth factor and basic fibroblast growth factor added to culture media under gravity and simulated microgravity. Tissue Eng Part A 16(5):1559–1573. doi:10.1089/ten.TEA.2009.0524

Grimm D, Wehland M, Pietsch J, Aleshcheva G, Wise P, van Loon J, Ulbrich C, Magnusson NE, Infanger M, Bauer J (2014) Growing tissues in real and simulated microgravity: new methods for tissue engineering. Tissue Eng Part B Rev 20(6):555–566. doi:10.1089/ten.TEB.2013.0704

Grosse J, Wehland M, Pietsch J, Schulz H, Saar K, Hubner N, Eilles C, Bauer J, Abou-El-Ardat K, Baatout S, Ma X, Infanger M, Hemmersbach R, Grimm D (2012) Gravity-sensitive signaling drives 3-dimensional formation of multicellular thyroid cancer spheroids. FASEB J 26(12):5124–5140. doi:10.1096/fj.12-215749

Halfter K, Hoffmann O, Ditsch N, Ahne M, Arnold F, Paepke S, Grab D, Bauerfeind I, Mayer B (2016) Testing chemotherapy efficacy in HER2 negative breast cancer using patient-derived spheroids. J Transl Med 14(1):112. doi:10.1186/s12967-016-0855-3

Hammond TG, Hammond JM (2001) Optimized suspension culture: the rotating-wall vessel. Am J Physiol Renal Physiol 281(1):F12–F25

Hollister SJ (2005) Porous scaffold design for tissue engineering. Nat Mater 4(7):518–524. doi:10.1038/nmat1421

Hwang YS, Cho J, Tay F, Heng JY, Ho R, Kazarian SG, Williams DR, Boccaccini AR, Polak JM, Mantalaris A (2009) The use of murine embryonic stem cells, alginate encapsulation, and rotary microgravity bioreactor in bone tissue engineering. Biomaterials 30(4):499–507. doi:10.1016/j.biomaterials.2008.07.028

Infanger M, Kossmehl P, Shakibaei M, Baatout S, Witzing A, Grosse J, Bauer J, Cogoli A, Faramarzi S, Derradji H, Neefs M, Paul M, Grimm D (2006) Induction of three-dimensional assembly and increase in apoptosis of human endothelial cells by simulated microgravity: impact of vascular endothelial growth factor. Apoptosis 11(5):749–764. doi:10.1007/s10495-006-5697-7

Ingram M, Techy GB, Saroufeem R, Yazan O, Narayan KS, Goodwin TJ, Spaulding GF (1997) Three-dimensional growth patterns of various human tumor cell lines in simulated microgravity of a NASA bioreactor. In Vitro Cell Dev Biol Anim 33(6):459–466. doi:10.1007/s11626-997-0064-8

Jessup JM, Goodwin TJ, Spaulding G (1993) Prospects for use of microgravity-based bioreactors to study three-dimensional host-tumor interactions in human neoplasia. J Cell Biochem 51(3):290–300. doi:10.1002/jcb.240510308

Jin F, Zhang Y, Xuan K, He D, Deng T, Tang L, Lu W, Duan Y (2010) Establishment of three-dimensional tissue-engineered bone constructs under microgravity-simulated conditions. Artif Organs 34(2):118–125. doi:10.1111/j.1525-1594.2009.00761.x

Klaus DM (2001) Clinostats and bioreactors. Gravit Space Biol Bull 14(2):55–64

Kopp S, Warnke E, Wehland M, Aleshcheva G, Magnusson NE, Hemmersbach R, Corydon TJ, Bauer J, Infanger M, Grimm D (2015) Mechanisms of three-dimensional growth of thyroid cells during long-term simulated microgravity. Sci Rep 5:16691. doi:10.1038/srep16691

Langer R, Vacanti JP (1993) Tissue Engineering. Science 260(5110):920–926

Lappa M (2003) Organic tissues in rotating bioreactors: fluid-mechanical aspects, dynamic growth models, and morphological evolution. Biotechnol Bioeng 84(5):518–532. doi:10.1002/bit.10821

Liphardt AM, Mundermann A, Koo S, Backer N, Andriacchi TP, Zange J, Mester J, Heer M (2009) Vibration training intervention to maintain cartilage thickness and serum concentrations of cartilage oligometric matrix protein (COMP) during immobilization. Osteoarthr Cartil 17(12):1598–1603. doi:10.1016/j.joca.2009.07.007

Luo W, Xiong W, Qiu M, Lv Y, Li Y, Li F (2011) Differentiation of mesenchymal stem cells towards a nucleus pulposus-like phenotype utilizing simulated microgravity in vitro. J Huazhong Univ Sci Technolog Med Sci 31(2):199–203. doi:10.1007/s11596-011-0252-3

Lv Q, Nair L, Laurencin CT (2009) Fabrication, characterization, and in vitro evaluation of poly(lactic acid glycolic acid)/nano-hydroxyapatite composite microsphere-based scaffolds for bone tissue engineering in rotating bioreactors. J Biomed Mater Res A 91(3):679–691. doi:10.1002/jbm.a.32302

Ma X, Wehland M, Schulz H, Saar K, Hubner N, Infanger M, Bauer J, Grimm D (2013) Genomic approach to identify factors that drive the formation of three-dimensional structures by EA.hy926 endothelial cells. PLoS One 8(5):e64402. doi:10.1371/journal.pone.0064402

Ma X, Pietsch J, Wehland M, Schulz H, Saar K, Hubner N, Bauer J, Braun M, Schwarzwalder A, Segerer J, Birlem M, Horn A, Hemmersbach R, Wasser K, Grosse J, Infanger M, Grimm D (2014) Differential gene expression profile and altered cytokine secretion of thyroid cancer cells in space. FASEB J 28(2):813–835. doi:10.1096/fj.13-243287

Marlovits S, Tichy B, Truppe M, Gruber D, Vecsei V (2003) Chondrogenesis of aged human articular cartilage in a scaffold-free bioreactor. Tissue Eng 9(6):1215–1226. doi:10.1089/10763270360728125

Mehta G, Hsiao AY, Ingram M, Luker GD, Takayama S (2012) Opportunities and challenges for use of tumor spheroids as models to test drug delivery and efficacy. J Control Release 164(2):192–204. doi:10.1016/j.jconrel.2012.04.045

Nishikawa M, Ohgushi H, Tamai N, Osuga K, Uemura M, Yoshikawa H, Myoui A (2005) The effect of simulated microgravity by three-dimensional clinostat on bone tissue engineering. Cell Transplant 14(10):829–835

Ohyabu Y, Kida N, Kojima H, Taguchi T, Tanaka J, Uemura T (2006) Cartilaginous tissue formation from bone marrow cells using rotating wall vessel (RWV) bioreactor. Biotechnol Bioeng 95(5):1003–1008. doi:10.1002/bit.20892

Pietsch J, Sickmann A, Weber G, Bauer J, Egli M, Wildgruber R, Infanger M, Grimm D (2011) A proteomic approach to analysing spheroid formation of two human thyroid cell lines cultured on a random positioning machine. Proteomics 11(10):2095–2104. doi:10.1002/pmic.201000817

Pietsch J, Ma X, Wehland M, Aleshcheva G, Schwarzwalder A, Segerer J, Birlem M, Horn A, Bauer J, Infanger M, Grimm D (2013) Spheroid formation of human thyroid cancer cells in an automated culturing system during the Shenzhou-8 space mission. Biomaterials 34(31):7694–7705. doi:10.1016/j.biomaterials.2013.06.054

Qiu Q, Ducheyne P, Gao H, Ayyaswamy P (1998) Formation and differentiation of three-dimensional rat marrow stromal cell culture on microcarriers in a rotating-wall vessel. Tissue Eng 4(1):19–34. doi:10.1089/ten.1998.4.19

Ravi M, Ramesh A, Pattabhi A (2016) Contributions of 3D cell cultures for cancer research. J Cell Physiol. doi:10.1002/jcp.25664

Riwaldt S, Bauer J, Pietsch J, Braun M, Segerer J, Schwarzwalder A, Corydon TJ, Infanger M, Grimm D (2015a) The importance of caveolin-1 as key-regulator of three-dimensional growth in thyroid cancer cells cultured under real and simulated microgravity conditions. Int J Mol Sci 16(12):28296–28310. doi:10.3390/ijms161226108

Riwaldt S, Pietsch J, Sickmann A, Bauer J, Braun M, Segerer J, Schwarzwalder A, Aleshcheva G, Corydon TJ, Infanger M, Grimm D (2015b) Identification of proteins involved in inhibition of spheroid formation under microgravity. Proteomics 15(17):2945–2952. doi:10.1002/pmic.201500067

Riwaldt S, Bauer J, Wehland M, Slumstrup L, Kopp S, Warnke E, Dittrich A, Magnusson NE, Pietsch J, Corydon TJ, Infanger M, Grimm D (2016) Pathways regulating spheroid formation of human follicular thyroid cancer cells under simulated microgravity conditions: a genetic approach. Int J Mol Sci 17(4):528. doi:10.3390/ijms17040528

Saltzman WM (1997) Weaving cartilage at zero g: the reality of tissue engineering in space. Proc Natl Acad Sci U S A 94(25):13380–13382

Sanford GL, Ellerson D, Melhado-Gardner C, Sroufe AE, Harris-Hooker S (2002) Three-dimensional growth of endothelial cells in the microgravity-based rotating wall vessel bioreactor. In Vitro Cell Dev Biol Anim 38(9):493–504. doi:10.1290/1071-2690(2002)038<0493:tgoeci>2.0.co;2

Schwarz RP, Goodwin TJ, Wolf DA (1992) Cell culture for three-dimensional modeling in rotating-wall vessels: an application of simulated microgravity. J Tissue Cult Methods 14(2):51–57

Sikavitsas VI, Bancroft GN, Mikos AG (2002) Formation of three-dimensional cell/polymer constructs for bone tissue engineering in a spinner flask and a rotating wall vessel bioreactor. J Biomed Mater Res 62(1):136–148. doi:10.1002/jbm.10150

Song K, Yang Z, Liu T, Zhi W, Li X, Deng L, Cui Z, Ma X (2006) Fabrication and detection of tissue-engineered bones with bio-derived scaffolds in a rotating bioreactor. Biotechnol Appl Biochem 45(Pt 2):65–74. doi:10.1042/ba20060045

Song KD, Liu TQ, Li XQ, Cui ZF, Sun XY, Ma XH (2007) Three-dimensional expansion: in suspension culture of SD rat's osteoblasts in a rotating wall vessel bioreactor. Biomed Environ Sci 20(2):91–98

Song K, Liu T, Cui Z, Li X, Ma X (2008) Three-dimensional fabrication of engineered bone with human bio-derived bone scaffolds in a rotating wall vessel bioreactor. J Biomed Mater Res A 86(2):323–332. doi:10.1002/jbm.a.31624

Stamenkovic V, Keller G, Nesic D, Cogoli A, Grogan SP (2010) Neocartilage formation in 1 g, simulated, and microgravity environments: implications for tissue engineering. Tissue Eng Part A 16(5):1729–1736. doi:10.1089/ten.tea.2008.0624

Strollo F (1999) Hormonal changes in humans during spaceflight. Adv Space Biol Med 7:99–129

Tschopp A, Cogoli A, Lewis ML, Morrison DR (1984) Bioprocessing in space: human cells attach to beads in microgravity. J Biotechnol 1:287–293

Ulbrich C, Westphal K, Pietsch J, Winkler HD, Leder A, Bauer J, Kossmehl P, Grosse J, Schoenberger J, Infanger M, Egli M, Grimm D (2010) Characterization of human chondrocytes exposed to simulated microgravity. Cell Physiol Biochem 25(4–5):551–560. doi:10.1159/000303059

Ulbrich C, Wehland M, Pietsch J, Aleshcheva G, Wise P, van Loon J, Magnusson N, Infanger M, Grosse J, Eilles C, Sundaresan A, Grimm D (2014) The impact of simulated and real microgravity on bone cells and mesenchymal stem cells. Biomed Res Int 2014:928507. doi:10.1155/2014/928507

van Loon JJWA (2007) Some history and use of the random positioning machine, RPM, in gravity related research. Adv Space Res 39(7):1161–1165. doi:10.1016/j.asr.2007.02.016

Wang C, Tang Z, Zhao Y, Yao R, Li L, Sun W (2014) Three-dimensional in vitro cancer models: a short review. Biofabrication 6(2):022001. doi:10.1088/1758-5082/6/2/022001

Wang JZ, Zhu YX, Ma HC, Chen SN, Chao JY, Ruan WD, Wang D, Du FG, Meng YZ (2016) Developing multi-cellular tumor spheroid model (MCTS) in the chitosan/collagen/alginate

(CCA) fibrous scaffold for anticancer drug screening. Mater Sci Eng C Mater Biol Appl 62:215–225. doi:10.1016/j.msec.2016.01.045

Warnke E, Pietsch J, Wehland M, Bauer J, Infanger M, Gorog M, Hemmersbach R, Braun M, Ma X, Sahana J, Grimm D (2014) Spheroid formation of human thyroid cancer cells under simulated microgravity: a possible role of CTGF and CAV1. Cell Commun Signal 12:32. doi:10.1186/1478-811x-12-32

Wu X, Li SH, Lou LM, Chen ZR (2013) The effect of the microgravity rotating culture system on the chondrogenic differentiation of bone marrow mesenchymal stem cells. Mol Biotechnol 54(2):331–336. doi:10.1007/s12033-012-9568-x

Wuest SL, Richard S, Kopp S, Grimm D, Egli M (2015) Simulated microgravity: critical review on the use of random positioning machines for mammalian cell culture. Biomed Res Int 2015:971474. doi:10.1155/2015/971474

Yu B, Yu D, Cao L, Zhao X, Long T, Liu G, Tang T, Zhu Z (2011) Simulated microgravity using a rotary cell culture system promotes chondrogenesis of human adipose-derived mesenchymal stem cells via the p38 MAPK pathway. Biochem Biophys Res Commun 414(2):412–418. doi:10.1016/j.bbrc.2011.09.103

Zhang X, Nan Y, Wang H, Chen J, Wang N, Xie J, Ma J, Wang Z (2013) Model microgravity enhances endothelium differentiation of mesenchymal stem cells. Naturwissenschaften 100(2):125–133. doi:10.1007/s00114-012-1002-5