Microgravity Effects on the Matrisome

Subjects: Cell Biology

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Gravity is fundamental factor determining all processes of development and vital activity on Earth. During evolution, a complex mechanism of response to gravity alterations was formed in multicellular organisms. It includes the “gravisensors” in extracellular and intracellular spaces. Inside the cells, the cytoskeleton molecules are the principal gravity-sensitive structures, and outside the cells these are extracellular matrix (ECM) components. The cooperation between the intracellular and extracellular compartments is implemented through specialized protein structures, integrins. The gravity-sensitive complex is a kind of molecular hub that coordinates the functions of various tissues and organs in the gravitational environment. The functioning of this system is of particular importance under extremal conditions, such as spaceflight microgravity.

Keywords: space flights ; microgravity ; matrisome ; connective tissues ; stromal lineage cells ; transcriptomics ; proteomics

1. Introduction

In recent decades, significant progress has been made in the understanding of basic principles of the cellular response to the alterations of gravitational stimulus due to the development of cellular and molecular biology approaches [1][2][3][4].

According to the current mechanochemical hypothesis, integrins and other cell surface receptors play an important role in the physical interaction between extracellular matrix (ECM) and cytoskeleton. Mechanically forced deformations in these linked structures switch on/off various intracellular molecular events involving the cytoskeleton structures and associated signal transduction cascades [4][5][6][7].

ECM, as a counterpart of the gravisensivity network, remains much less studied than the cell surface structures, cytoskeleton and related intracellular events. At the same time, it is well known that the skeleton with abundant ECM as well as muscle tissues are most sensitive to space flight microgravity [2][8][9][10][11]. It is obvious that progress in the study of the response to microgravity and the development of approaches to the prevention of the negative effects of gravity deprivation is impossible without considering the role of ECM.

2. Connective Tissue Matrisome and Microgravity

2.1. Space Flight Experiments

It is well established that a significant decrease in bone mineral density resulting in osteopenia is one of the most pronounced effects of gravity deprivation in humans and animals [8][9]. The severity of the observed bone microstructure rearrangement was found to largely depend on the bone location relative to the gravity vector [8][12]. The local bone mass loss under mechanical stress deficiency or microgravity suggests that the mechanical signal reception (or its absence) can also be performed at the cellular level. Other organs with a well-developed connective tissue component, such as muscles, blood vessels, and skin [13], can also undergo negative changes under the influence of microgravity, including atrophy [14].

The mechanisms of gravisensitivity of stromal lineage cells of different commitment in the above connective tissue compartments are being intensively studied. At a moment, the attention is mainly focused on the molecular cascades associated with the mechanotransduction from the extracellular space to the cytoskeleton [4][5][6][7]. The involvement of matrisome as a gravireceptor has not yet been adequately studied. Meanwhile, the already existing data elicit some changes in the main matrisome compartments.

The opportunities to execute the experiments on board of unmanned or manned space vehicles are extremely limited. Therefore, a few ones have obtained data on the changes of the matrisome elements during space flights. On ISS, a

single study on the whole organism - a medaka fish, was performed [15]. At flight day 1 live-imaging detected an excessive fluorescence of a core glycoprotein DsRed-osteocalcin in pharyngeal bone osteoblasts versus ground-based control. At flight days 5 and 8, the increase fluorescent signal was sustained. High throughput sequencing analysis of pharyngeal bones of juvenile fish at day 2 after launch detected upregulation of two osteoblast-related genes *COL10A1* and osteocalcin (*OCN*) as well as ECM-remodeling *MMP9* [15].

In several space missions, in vitro experiments of different duration were performed using the osteogenic precursors (**Table 1**). Both in Foton 10 satellite unmanned [16] and STS-54 [17], STS-59 [18], STS-65 [19] manned missions a downregulation of *OCN* starting from flight day 5 till day 12 was detected. In addition, experiments at Foton 10 and STS-59 demonstrated a downregulation of the master core protein, *COL1A*. In a recent experiment at SJ-10, downregulation of several genes encoded core matrisome and upregulation of *MMP1* was observed after 2 days of flight. The inhibiting effect on *COL1A2* was detected in the same mission after 5 days [20]. Based on inflight observations, it can be assumed that microgravity negatively affects core matrisome proteins at the transcriptional level. The increased DsRed-osteocalcin protein fluorescent signal described in medaka fish may indicate the post-translational effects of microgravity.

Table 1. The effects of real microgravity and its simulation on matrisome components of stromal lineage cells.

Object	Cell Type	Duration	Matrisome			Exp. Approach	Study
			Core Matrisome		ECM-Associated Molecules		
			Collagens	Proteoglycans, Glycoproteins			
Space flight							
Medaka fish	OB, Ocl (ISS)	1, 5, 8 d	U: *COL10A1*	U: *OCN*	U: *MMP9*	DsRed live-imaging, GGA	[15]
Mouse	MG-63, osteoinduced (Foton 10)	9 d	D: *COL1A;* ND: collagen type I	D: *OCN*		RT-PCR, WB	[16]
Mouse	2T3 OB (STS-54)	6 d		D: *OCN*		RT-PCR	[17]
Chicken	OB (STS-59)	12 d	D: *COL1A;* ND: collagen type I	D: *OCN*		RT-PCR, WB	[18]
Rat	OB (STS-65)	5 d		D: *OCN*		RT-PCR	[19]
Human	OB, MSC-derived (SJ-10)	2 d	D: *COL1A1, -1A2, -3A1, -4A1, -5A1, -6A1, -8A1*	D*: TNC, COMP*	U*: MMP1*	RT-PCR	[20]
Human	OB, MSC-derived (SJ-10)	5 d	D: *COL1A1*			RT-PCR	[20]
Hind-limb suspension (HS)							
Rat	MSC-BM, tibia	5 d		D: *OCN*		RT-PCR	[17]
Rat	MSC-BM, tibia	14 d		U: *OCN*		RT-PCR	[17]
Rat	MSC-BM, femur	28 d		D: *RUNX2, COLI, ALP, OCN*	D: osteogenic potential; expression of osteoblast gene marker mRNAs under osteogenic conditions.	RT-PCR, HS	[21]
Mouse	MSC-BM, femur	7 d			D: mineralization	HS	[22]
Rotating-Wall Vessel (RWV)							

Object	Cell Type	Duration	Matrisome			Exp. Approach	Study
			Core Matrisome		ECM-Associated Molecules		
			Collagens	Proteoglycans, Glycoproteins			
Human	MSC-BM	7 d	D*: COL2A1, COL10A1*	D*: ON*		RT-PCR	[23]
Mouse	OB MC3T3-E1	1–14 d	ND: *COL1A2*	ND: *OCN*		RT-PCR	[23]
Mouse	OB/OC MLO-A5	1–14 d	ND: *COL1A3*	ND: *OCN, OPN*		RT-PCR	[24]
Mouse	MSC C3H10T(1/2)	1–14 d	ND*: OCN, OPN, COL1A4*	ND: *OCN, OPN*		RT-PCR	[24]
Mouse	OB MC3T3-E1	1 d		D*: OCN*		RT-PCR	[25]
Human	MG-63, osteoinduced	3 d	D: *COL1*	D*: OCN*		RT-PCR	[26]
Human	ChB, MSC-derived	21 d	D: *COL1*	D: *AGN*		RT-PCR	[27]
Mouse	OB	1d	ND: *COL1A2*	ND: *OCN, OPN*		RT-PCR	[28]
			Random Positioning Machine (RPM)				
Human	FB	3 d	U: *COL4A5*	U*: FN*/FN	U*: TGF*	RT-PCR, WB	[3]
Human	MSC-AT	4 d	U: *COL12A1, COL15A1, COL16A1, COL1A1, COL5A1, COL8A1*	U: *THBS1, THBS2, THBS3, LAMA, SPARC, TNC, VCAN, VTN; D: CLEC3B*		RT-PCR	[29]
Human	MSC-AT	10 d	D: *COL11A1;* D*:* collagenous proteins	D*: LAMB3, TNC;* U*:* non-collagenous proteins		RT-PCR, HC	[30]
Human	MSC-BM, osteoinduced	20 d	U: *COL1A1*	D: *OMD;* ND*: OCN*	D: ECM mineralization	RT-PCR, HC	[31]
Human	MSC-BM	5 d	D: *COL9A1, COL2A1*			RT-PCR	[32]
Human	MSC-BM, osteoinduced	10 d	ND*: COL1A1*	D*: OMD;* ND*: OCN*		RT-PCR, ICC	77]
Human	MSC-BM	20 d	ND: *COL1A1*; ND: collagen tot			RT-PCR, ICC	[32]
Human	OB	20 d			U: ECM mineralization	HC	[32]
Mouse	2T3 OB	3 d		D: *OMD*		RT-PCR	[33]
			2D, 3D-clinorotation				
Mouse	MC3T3-E2	3 d			U: *PLOD1, PLOD2;* U: enzymes activity	RT-PCR, enzyme assay	[34]
Human	MSC-AT	7 d	U*: COL1 COL3*	D: *FBN1*	D: *MMP1*	RT-PCR	[35]
Mouse	MC3T3-E1	7 d	D: *COL1A1*			RT-PCR	[36]
Rat	MSC	1–4 d			D: *cbfa1/RUNX2*	RT-PCR	[37]

Abbreviations: cell types: MSC—mesenchymal stromal cell; OB—osteoblast; OC—osteocyte; ChB—chondroblast; OCl—osteoclast. MSC sources: BM—bone marrow; AT—adipose tissue. Experimental approaches: RT-PCR; ICC—immunocytochemistry; HC—histochemistry; WB—Western blot; GGA—Hiseq global gene analysis. Matrisome and matrisome-associated molecules: COL—collagen, FN—fibronectin, FBN1—fibrillin; LAMA—laminin; OMD—osteomodulin; cbfa1/RUNX2—master transcription factor of osteogenic differentiation; OCN (BGLAP)—osteocalcin; ON (SPARC)—osteonectin; OPN (SPP1)—osteopontin; AGN—aggrecan; THBS—trombospondin; TNC—tenascin, VCAN—versican; VTN—vitronection; TNC—tenascin. The direction of the effects: D—downregulation; U—upregulation; ND—no difference.

2.2. Ground-Based Simulations

Various approaches have been developed to simulate spaceflight effects under ground-based conditions, including experiments at the physiological level or individual cells (**Table 1**).

In animal experiments, anti-orthostatic suspension of rodents (mice or rats) is the mostly demanded “unloading” model. In several studies, low-committed stromal precursors (MSCs) were isolated from the hind limb bone marrow from suspended and control animals. The subsequent cultivation demonstrated a decreased osteogenic potential of MSCs associated with a downregulation of osteoblastic commitment-related genes [21], including core glycoprotein ostepontin (*OPN*), and a decreased mineralization of the ECM [22]. It is important to note that after a short-term suspension (5 days), a decreased *OCN* transcription was detected in cultured MSCs [17], which was consistent with the effects described in osteo-precursor cultures in spaceflights. The same authors found an increased *OCN* expression in MSCs isolated and expanded after 14 days of suspension [17]. Since the multidirectional changes in transcription of *OCN* were described in the same experiment, this may be an indication of time-dependent response of ECM-associated genes.

To study the effects of microgravity on the cells, various devices have been developed to simulate the absence of gravity. These include devices that provide fast and slow rotations of biological objects (2D and 3D clinorotation), three-dimensional dynamic rotation (Random Positioning Machine (RPM), and large-volume rotating-wall vessels (RWV) [38]. All ground-based simulations provide a randomization of position of cells relative to the gravity vector. The limitations in each case do exist. 1D/2D clinostat/RWV cancel the directionality of the gravity vector attenuating but not eliminating the gravity. The shift in weight distribution can cause mechanical and bending stress. Using RPM, high quality microgravity conditions down to 10^{-4} g can be obtained. It strongly depends on the combination of rotation speed and the distance from the center, thus, the experimental conditions must be carefully set [39].

Such devices provide the opportunity to identify the mechanisms of influence of the altered gravitational environment on cells as well as to adapt methodological approaches before using them in the spaceflights.

RWV experiments of various durations showed no change or a decrease in the transcription of the main core collagen I and a number of core glycoproteins (**Table 1**). These effects were similar in the low committed [23][24] or osteo/chondro-induced stromal progenitors [25][26][27], osteoblasts [28], and osteocytes [24].

Using gravity vector randomization approaches with 2D/3D clinostats or RPM devices, multidirectional changes in the transcription of core protein genes and ECM-associated and affiliated molecules were found (**Table 1**).

An upregulation of genes encoding core matrisome proteins was demonstrated in a number of papers [3][29][30][31][32][33][34][35][36][37]. In juvenile human fibroblasts, 3 days SMG (RPM) induced an increase of transcription of core basement membrane collagen IV, and transcription/translation of the principal core glycoprotein fibronectin, as well as of MMPs involved in ECM remodeling [3]. Upregulation of several genes encoding core proteins and glycoproteins in human adipose MSCs was described after 4 days of RPM exposure [29]. Though RPM exposure till day 10 affected fewer differentially expressed matrisome genes, a part of core matrisome encoded genes were still significantly upregulated, while genes encoding ECM-degrading enzyme and its inhibitors were downregulated [30].

Besides, it was demonstrated a time-dependent dynamic of the transcriptional activity of matrisome genes under simulated microgravity exposure [31][32]. In comparison to static conditions, core collagens were downregulated in bone marrow MSCs after 5 days at RPM, there were no differences after 10 days, and these genes were significantly upregulated after 20 days. At the same time, after 10 and 20 days of exposure, the expression of core glycoprotein osteomodulin (*OMD*) that regulates osteoblast adhesion was reduced as well as ECM-associated growth factors, while *OCN* transcription did not change [32]. Regardless of exposure time, a decreased transcription of *OMD* was demonstrated in human murine and preosteoblasts [32][33].

Long-term microgravity simulation with RPM was demonstrated to have different effects on the efficacy of mineralization of the ECM by stromal cells of different commitment levels. In osteocommitted MSCs, the deposition of calcium was reduced, and in osteoblasts, on the contrary, it was increased [32].

In MC3T3-E2 lineage osteoblasts, 3 days of 3D-clinorotation was accompanied by upregulation of genes encoding enzymes that provide extracellular posttranslational modification of collagen fibers, and the increase of functional activity of the above enzymes [34]. An increased expression of core collagen gene was detected after 7 days of human adipose MSC 2D clinorotation [35].

On the other hand, the data are available on the suppressive effects of simulated microgravity on matrisome compartments. The downregulation of glycoprotein fibrillin (*FBN1*) and *MMP1* was noted after 7 days 2D clinorotation of human adipose MSCs [35] as well as of core *COL1* and *FBN1* in MC3T3-E2 murine osteoblasts following 7 days of 3D-clinorotation [36]. In addition to changes in core proteins, a decreased transcriptional activity of genes encoding molecules associated with ECM metabolism like transcription factors *cbfa1/RUNX2* was noted [37].

Thus, the available in vivo and ex vivo information on the effects of real and simulated microgravity on the ECM and related molecules indicate the direct involvement of the matrisome components in the adaptation of stromal lineage cells to gravity deprivation. The short-term exposures provoke multidirectional alterations in ECM-related gene activity, which ensures the adaptation. In general, the direction of changes of the matrisome gene transcription does not change critically depending on the commitment level of stromal progenitors. However, the above data by Gershovich et al. have shown that the differences in the ECM response can be reflected at the ECM maturation level [32]. In this regard, the search for integral markers that make it possible to assess the changes in the body matrisome under various extremal conditions is becoming more and more demanded. The application of rapidly developing omics is one of the most promising approaches in the above direction.

3. Proteomic Profile of Human Matrisome-Associated Proteins under Real and Simulated Microgravity

Proteomic approach attracts considerable attention in the study of physiological and pathological changes. Several recent reviews describe the effect of microgravity on the animal and human proteomes [40][41][42][43][44]. Over the past few years, mass spectrometry has become the method of choice for the characterization of ECM composition [45][46][47] and has been shown to offer new bioinformatic approaches of translating data from the putative biomarkers to the elucidation of new therapeutic targets [48][49][50]. The experimental strategies, new bioinformatic tools, and methods for matrix isolation have been described for the research on the ECM composition and mechanisms of degradation/renewal [45][51][52][53][54]. The new MatrisomeDB version contains selected proteomic data from 17 studies with ECM from 15 various tissues and includes 847 human ECM proteoforms and over 350,000 peptide-to-spectrum matches [52].

The proteomic data on matrisome components are considered to be of diagnostic and prognostic values in clinical studies. A comparison of core ECM and ECM-associated molecules' data from the human carotid endarterectomy samples demonstrated the differences in the proteome and gene expression in symptomatic and asymptomatic atherosclerotic patients, including MMP-9, chitinase 3-like-1, calcium binding protein S100 A8 (S100A8), S100A9, cathepsin B, fibronectin, and galectin-3-binding protein [55]. Proteomic analysis revealed the loss of aggrecan and several small leucine-rich proteoglycans, with a compensatory increase in collagen I during ECM remodeling in varicose veins, though there were no significant alterations of gene expression. These data suppose that the remodeling process associated with venous hypertension mainly occurs at the translation level, rather than at the transcription one. [48].

Investigation of the effects of spaceflights and ground-based simulations on the proteomics of matrisome in healthy subjects is of great interest. Several experimental modes that involve healthy volunteers are used to simulate the effects of certain spaceflight factors. These include various types of immersion and head-down tilt bed rest (HDT BR).

After 21 days of "dry" immersion, the altered levels of a number of proteins were detected in plasma with chromatography–mass spectrometric analysis. The identification of overrepresented processes, as well as processes and biological pathways was performed using the GO databases (biological processes, pathways, and KEGG). The significantly changed proteins were annotated as involved in ECM remodeling (alpha, beta, fibrinogen gamma chains), fibronectin, transtyretin), vitronectin and the cell morphogenesis regulation (alipoprotein A-I, prothrombin, alpha, beta, gamma chains of fibrinogen, fibronectin [56][57]. Hypokinesia is accompanied "dry' immersion as well, and probably causes a protease/counter protease imbalance, which may be responsible for the ECM remodeling activation. Besides, after 21 day "dry" immersion a decrease in ECM proteoglycans, lumican and COMP, was also detected. COMP, as a cartilage

structural protein, plays an important role in the ECM stabilization due to the interactions with collagen fibrils and other matrix components [58].

The blood COMP level is sensitive to physiological stress. The study of 14 day HDT BR demonstrated that the joint cartilage thickness was reduced during the experiment, followed by a decrease in the COMP level [59]. Liu et al. [60] reported the chondrocytes' ability to respond to stress in the extracellular environment (possibly both mechanical and shear stress), which resulted in an altered expression of matrix proteins. Therefore, a decrease in the blood COMP level during "dry" immersion may reflect a reduced metabolic activity of cartilage matrix proteins in response to the lack of mechanical stimuli.

Based on the proteomic data of blood and urine samples collected in HDT BR and "dry" immersion of similar durations (21 days), GO pathways analysis was performed for proteins with significantly changed concentrations. It was demonstrated that ECM remodeling was the most significant process among them (in particular, collagen degradation) [61]. At day 21 of HDT BR, a significant decrease was observed in the levels of collagen I and XV alpha-chains, and cathepsin D that are associated with degradation processes and ECM collagen fibril assembly.

According to the "dry" immersion and HDT BR proteomic data comparison, at day 21, proteins involved in the ECM organization and metabolism were detected: endorepellin, nidogen-1, tenascin X, and vitronectin. Bioinformatic resources confirm that proteins endorepellin and nidogen-1 are primarily involved in the ECM degradation. The analysis demonstrated that the proteins that changed their blood levels under HDT BR have the catalytic activity functions (transferase and hydrolase activities). These findings provide information about the ECM structures' involvement in the response to a reduced support load.

Urine, blood, and exhaled air condensate are the minimally invasive biological material samples available for proteomic research in astronauts. Researchers believe that blood samples are the most preferable ones for the study of ECM components among the above liquids. da Silveira et al. have applied a multidisciplinary systemic biology analytical approach to determine transcriptomic, proteomic, metabolomic, and epigenetic responses to spaceflight [62]. Multi-omics datasets obtained from the analysis of an astronaut's biological samples after a one-year space mission showed a significant enrichment of biological processes closely related to the functions of ECM.

Blood samples obtained from the Russian cosmonauts were examined by various proteomic mass spectrometry-based methods. Changes in the serum protein composition, including full-size proteins and the isoforms, fragments, metabolites, and peptides, after long-term spaceflights were characterized by direct mass spectrometry profiling after serum pre-fractioning using MB WCX magnetic particles. After a spaceflight, the peak areas of "acute phase" proteins, lipid metabolism, proteolytic enzymes and their inhibitors were shown to alter [63].

With semi-quantitative label free panoramic method, it was demonstrated that among 419 various proteins in cosmonauts' blood plasma 17 proteins were significantly increased, while two significantly decreased after a prolonged space flight in comparison with pre-flight levels. In most cases, these proteins do not return to pre-flight baselines by day 7 after spaceflight. They are involved in the blood clotting system, ECM remodeling, and immune processes [64].

The quantitative changes in the cosmonauts' blood proteome found using targeted MRM method with a panel of stable [^{13}C]/[^{15}N] isotope-labeled proteotypic peptides indicated that almost all proteins with the concentrations reacting to space flight can be combined into a network of interactions between the processes of regulation of protease activity, innate immunity, lipid metabolism, coagulation cascades, and ECM metabolism [43]. Latridis et al. suggest that these reactions may be triggered by extracellular signaling pathways of mechanotransduction [65]. Part of the group of functionally ECM-associated proteins detected in the samples at day 1 after landing in a reduced concentration, were found to return to baseline after 7 days, but another part of proteins retained significantly reduced concentrations [63][64]. The ANDCell program made it possible to identify biological processes involving ECM proteins that are modified by spaceflight factors (**Table 2**).

Table 2. Spaceflight factors-affected biological processes involving matrisome proteins.

Protein	Uniprot Index	Biological Process (ANDCell)
Alpha-2-HS-glycoprotein	FETUA_HUMAN	Positive regulation of ECM constituent secretion; Regulation of ECM assembly; erk 1/2 mitogen-activated protein kinase pathway ANG 2; ANG2 expression of ECM proteins; ANG2 erk1/2 pathway; mek/erk pathway; erk pathway
Angiotensinogen	ANGT_HUMAN	erk pathway
Apolipoprotein A-I	APOA1_HUMAN	erk pathway
Apolipoprotein E	APOE_HUMAN	Positive regulation of ECM constituent secretion
Carboxypeptidase B2	CBPB2_HUMAN	erk pathway
Cathelicidin antimicrobial peptide	CAMP_HUMAN	ras-erk pathway
CD44 antigen	CD44_HUMAN	MMP9 signaling pathway; ras-erk1/2 pathway; mek/erk pathway; erk pathway.
Clusterin	CLUS_HUMAN	Inhibition of ECM disassembly; ECM organization; mapk/erk pathway; MMP9 signaling pathway
Cystatin-C	CYTC_HUMAN	ECM organization
Fibronectin	FINC_HUMAN	ECM organization; Activation of erk pathway; ECM assembly; erk1/2 pathway; Cell–matrix adhesion; erk pathway; Calcium independent cell matrix adhesion; mapk/erk pathway
Fibulin-1	FBLN1_HUMAN	Cell–matrix adhesion
Insulin-like growth factor-binding protein 3	IBP3_HUMAN	erk1/2 pathway
Intercellular adhesion molecule 1	ICAM1_HUMAN	erk1/2 pathway; mek/erk pathway; Cell–matrix adhesion
Kininogen-1	KNG1_HUMAN	Bradykinin in MMP secretion; ECM secretion; mapk/erk pathway
Lumican	LUM_HUMAN	ECM assembly
Pigment epithelium-derived factor	PEDF_HUMAN	MMP secretion; apoptotic signaling pathway; erk1/2 pathway
Transthyretin	TTHY_HUMAN	Apoptotic signaling pathway; erk1/2 pathway
Vitronectin	VTNC_HUMAN	ECM organization; Cell–matrix adhesion

As evidenced from **Table 2**, the reorganization of the matrix structure due to MMPs, the ERK pathway regulation, cell–matrix adhesion, secretion, and assembly of ECM structures are the principal biological processes affected by spaceflight.

Proteomic analysis of urine samples from Russian cosmonauts after six-month missions detected 20 of the 256 proteins with altered levels [43][66]. However, the concentrations of most of them were returned to pre-flight levels within 7 days of the post-flight rehabilitation. At day 1 upon landing, seven proteins involved in ECM remodeling were identified among the significantly changing proteins. According to their functions in the physiological processes, they belong to the proteins involved in the musculoskeletal system metabolism. Thus, the level of osteodifferentiation and bone mineralization associated osteopontin was increased at day 1 upon landing and did not return to baseline after a further 7 days. Interestingly, several proteins not detected in urine samples prior to flight were increased acutely in post-flight: alpha-1-antichymotrypsin, N-acetyl glycosamine-6-sulfatase, cystatin-M, collagen alpha-1(I) chain, and vitronectin, granulin, and LDH beta chain. The above proteins are known to belong to the group of ECM-associated biological processes [66].

Therefore, the secretion and assembly of the matrisome components, its remodeling activity, as well as the ECM/cells associations are affected by spaceflight, primarily by the reduction of mechanical stress under microgravity.

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