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Suture in Space: Preparation of an Experiment on the Healing of Sutured Wounds on Board the ISS

Monica Monici[a]*, Francesca Cialdai[a], Daniele Bani[b], Stefano Bacci[c], Lucia Morbidelli[d], Aleandro Norfini[e], Michele Balsamo[e], Jack van Loon[f], Daniela Grimm[g], Stephan Riwaldt[h], Giuseppe Coppola[i], Leonardo Surdo[j], Francesca Ferranti[k], Desirée Pantalone[b]

[a] *ASAcampus Joint Laboratory, ASA Res. Div. & Dept. of Experimental and Clinical Biomedical Sciences "Mario Serio", Univ. of Florence, Vle Pieraccini 6, Florence, Italy,* monica.monici@unifi.it, francesca.cialdai@unifi.it
[b] *Dept. of Experimental and Clinical Medicine, Univ. of Florence, Vle Pieraccini 6, Florence, Italy,* daniele.bani@unifi.it, desire.pantalone@unifi.it
[c] *Dept. of Biology, Univ. of Florence, Viale Pieraccini 6, Florence, Italy,* stefano.bacci@unifi.it
[d] *Dept. Life Sciences, Univ. of Siena, Via A. Moro 2, Siena, Italy,* lucia.morbidelli@unisi.it
[e] *Kayser Italia Srl, Via di Popogna 501, Livorno, Italy.* a.norfini@kayser.it, m.balsamo@kayser.it
[f] *DESC, ACTA-Vrije University Amsterdam, Amsterdam, The Netherlands,* j.vanloon@amsterdamumc.nl
[g] *Inst. of Biomedicine, Aarhus University, Wilhelm Meyers Allé 4, Aarhus C, Denmark,* dgg@biomed.au.dk.
[h] *Otto-von-Guericke University Clinic, Leipziger Str. 44, Magdeburg, Germany,* stefan.riwaldt@med.ovgu.de
[i] *Inst Microelectr. Microsystems, CNR Naples, Via Pietro Castellino, Naples, Italy* giuseppe.coppola@cnr.it
[j] *European Space Agency, NL-2200AG Noordwijk, The Netherlands,* leonardo.surdo@esa.int
[k] *Italian Space Agency, Via del Politecnico snc, Rome, Italy,* francesca.ferranti@asi.it

* Corresponding Author

Abstract

Wound healing (WH) is a process strictly regulated and highly conserved throughout evolution because it is indispensable for surviving injuries. On Earth WH has been studied in depth, nevertheless the role of mechanical factors in regulating the process and the mechanisms that, in adult mammals, lead to scarring instead of tissue regeneration are not well understood. In weightlessness WH has been poorly studied, and the effect of loading/unloading on the healing mechanisms is quite completely unknown. Preliminary studies showed microgravity-induced alterations in mechanisms underlying tissue repair.

The implementation of procedures and tools to manage emergency surgery, trauma, serious burns, wounds and sutures is mandatory for future human deep space exploration missions at distances which are incompatible with medical evacuation to Earth. Therefore, studies on WH in weightlessness are needed and they are also an unique opportunity for understanding healing mechanisms still not completely known.

The Suture in Space experiment, which will be performed on board the International Space Station (ISS), was selected by ESA (ESA-AO-ILSRA-2014) and supported by ASI in its development phase. It aims to study in weightlessness the behavior and healing of ex vivo sutured wound models prepared from skin and blood vessels biopsies derived from plastic and vascular surgery in healthy subjects.

The experiment preparation required intense research activity on ground in order to: i) standardize procedures for collection of biopsies, model preparation, tissue culturing and monitoring, postflight analysis of samples; ii) define the requirements for hardware development.

To ensure tissue viability throughout the in-flight experiment (4 weeks), we studied and developed a new tissue culture technique based on enriched culture media and a device able to model the physiological mechanical tension in the tissues and monitor its changes during WH, thus enabling the study of suture mechanical properties.

The culture technique and WH models developed for the Suture in Space experiment can be applied to study: i) mechanical properties of tissues, tissue constructs, wounds and sutures in different loading conditions; ii) the role of gravity in tissue repair; iii) the relationship between biochemical and mechanical factors in repair mechanisms; iv) the influence of mechanical factors on scar quality; v) the effectiveness of treatments promoting WH, when applied in different loading conditions.

The results of the experiment are expected to help in defining: i) strategies to manage wounds and promote healing in Space and on Earth; ii) suture techniques and materials to be used in space environment.

Keywords: (wound healing, surgical wounds, tissue viability, space exploration, ISS, weightlessness)

Acronyms/Abbreviations

ASI	Agenzia Spaziale Italiana
CNR	Consiglio Nazionale delle Ricerche
DCB	Double Cold Bag
DMEM	Dulbecco's Modified Eagle Medium
EC	Experiment Containers
ECM	Extracellular Matrix
EHW	Experiment Hardware
ESA	European Space Agency
EST	Experiment Sequence Test
EU	Experiment Unit
ISS	International Space Station
LEO	Low Earth Orbit
μg	Microgravity
NASA	National Aeronautics and Space Administration
PBS	Phosphate Buffered Saline
RPMI	Roswell Park Memorial Institute
SVT	Science Verification Test
WH	Wound Healing

1. Introduction

Regardless of the cause (surgery, trauma, diabetes, pressure, poor venous/arterial flow, burns, etc.) wounds and compromised WH are major concerns on Earth: they cause patients significant discomfort and drain the medical systems of an enormous amount of resources [1,2]. Defective healing can lead to delayed wound closure, chronic ulcers and fibrosis. Several factors can affect WH and its complications. Some of them, such as age, overweight and systemic diseases (e.g. diabetes), cannot be changed. Some others, such as wound contamination, emergency care and wound care, mechanical factors and non-physiological environments, can be controlled.

Efficient tissue repair mechanisms are of paramount importance to ensure the integrity of the organism, restore the protective barrier and thus enable survival. WH is a complex process that begins at the moment of injury and can continue for days, weeks or even months. The healing phases begin immediately after clotting and are classically referred to as inflammation, proliferation and remodeling. They are strictly regulated by a multitude of biochemical and physical factors, including mechanical forces. Mechanical factors affect healing by acting both at cellular and tissue levels. Sutures also play a mechanical action closing the wound, distributing tension along the suture lines, restoring and maintaining the tensile strength in the tissue. Therefore, mechanical stress at the wound site is important in determining the behavior of cell populations involved in tissue repair mechanisms and the healing progression.

Remarkable progress has been achieved in understanding molecular and cellular mechanisms of WH on Earth, although some of them remain elusive. WH in Space is even less known. Reports on the "state of the art" in space surgery included WH and sutures among the critical aspects which have been relatively poorly studied in weightlessness and need to be further investigated [3].

The likelihood of surgical emergencies, traumatic events, severe wounds and burns occurring during current human space missions is considered low. In any case, the current protocols for the management of these events foresee patient stabilization and rapid return to Earth. Future human space exploration programs could require long duration missions beyond the Low Earth Orbit (LEO), in which the likelihood of surgical emergencies and traumatic events may increase while the medical evacuation times to Earth might become too long. Therefore, wound management aboard space vehicles will be crucial to ensure survival, but little is known about whether and how weightlessness affects WH.

In vitro studies on healing mechanisms in real and modeled microgravity (μg) have shown that weightless conditions affect the behavior of cell populations involved in WH, such as fibroblasts and endothelial cells. Fibroblast activation, angiogenesis and production of extracellular matrix (ECM) molecules are altered as well as cell ability to adhere, migrate and respond to chemoattractants [4-6]. Moreover, changes in ECM composition could affect the suture-tissue interaction and, consequently, the mechanical properties of the suture. Studies conducted in animal models did not result in any definite conclusion, but most of them pointed to delayed and defective healing, mostly due to impairments in the inflammatory and remodelling phases [7,8]. To the best of our knowledge, there are no studies focusing on WH in astronauts, but some alterations caused by spaceflight, such as immune deficiency, increase in low grade inflammation, changes in hemorrhage progression, modifications in the skin microbiota could significantly affect the body's response to injury in Space [9,10].

Conducting WH experiments aboard the ISS (or other spacecrafts) is important to understand if and how spaceflight may affect the various phases of the healing process and to develop adequate countermeasures. Moreover, due to the altered gravity conditions, the space environment offers a unique opportunity for understanding the role of gravity and mechanical factors in tissue repair processes. Better knowledge about the regulatory role played by mechanical and gravitational factors could help answer questions still pending: for example, unravel the mechanisms of perfect regeneration in embryos, an ability that is lost as development proceeds [11].

The Suture in Space experiment, selected by ESA (ESA-AO-ILSRA-2014) and supported by ASI, has been conceived to study the behavior and healing of *ex vivo* sutured wound models in weightlessness and will be conducted aboard the ISS. These rather complex *ex vivo* models and the hardware developed for the Suture in Space experiment allow to measure mechanical forces at the wound site, monitor the behavior of the sutured wounds and analyze the mechanisms involved in tissue repair. The goal of the experiment is to gain information on:

1. how weightlessness affects the mechanisms of tissue repair and regeneration;
2. the role of gravity and mechanical stress in WH progression, scar quality and restoration of tensile strength in the tissue*;*
3. how the suturing materials and techniques can be adapted to weightless conditions, thus developing strategies to foster WH in Space;
4. how to improve wound management in order to promote WH avoiding fibrotic scars both on Earth and in Space.

This paper reports on the activities carried out to prepare the space experiment, from the experiment design to the development of ex vivo sutured wound models and a new tissue culture technique, and finally the Experiment Hardware (EHW) implementation.

2. Material and methods

2.1 Experiment design

The Suture in Space experiment has been designed to be conducted in an automatic way in the ESA Biolab facility inside the Columbus module on board the ISS. It aims to study in weightlessness the behavior and healing of sutured wound models prepared from biopsies of skin and vein vessels.

The human biopsies will be collected at Careggi Hospital, Florence, Italy, under informed consent and approval by the Local Ethics Committee. The biopsies will be stitched to frames specifically developed to model the physiological tensile strength in the tissues (see 2.2 and 2.4), placed in transport containers with modified RPMI-based culture medium (see 2.2) and transferred at the launch site, maintaining T= 4±2°C during the transport.

At the launch site, incisions will be performed on the tissues and then sutured in order to model sutured wounds (see 2.2). Each sample (skin or vein vessel biopsy stitched on dedicated frame) will be placed in the culture chamber of a newly developed bioreactor - or Experiment Unit (EU) - filled with modified DMEM-based culture medium (see 2.2). The tensile strength in the sutured tissue will be measured with a load cell, which is part of the EU, in order to have a baseline measurement on-ground. In total, 8 EUs will be prepared: 4 EUs with skin samples and 4 EUs with vein vessel samples. The 8 EUs will be integrated two by two in 4 Advanced Experiment Containers (AEC).

During experiment's handover to the launch authorities and upload to the ISS, samples will be kept in NASA Double Cold Bags (DCBs) at about 24°C.

Once the Experiment Hardware (EH) has arrived to the ISS, the astronaut will take a picture of the sutured wound models, which will be visible through a window located on the AEC cover. Then, the AECs will be inserted inside the Biolab set at a temperature of 32°C, and the experiment will start. The culture medium contained in the culture chamber will be refreshed every 3 days. In each EU, the tensile strength in the tissue sample will be monitored throughout the experiment by the load cell. After 4 days of incubation at 32°C, 2 AECs with their EUs will be removed from the Biolab and the astronaut will take pictures of the sutured wound models (2 skin samples and 2 vessel samples). Then, the AECs will be transferred to the cold stowage facility at -80°C. The other 2 AECs (with the remaining 2 skin and 2 vessel samples) will continue incubation for 5 days more and will then be removed after 9 days from the Biolab and treated as described above. Experiment timeline and temperature profile inside the AECs will be logged together with the tensile strength measurements. All the samples will be kept stored at -80°C until return to Earth.

During the transfer from the ISS to Earth and subsequent transport to the science team's laboratories, samples will be maintained at about -20°C. After retrieval, the samples will be processed and analysed for: suture morphology and ultrastructure, blood vessels and endothelial function in the sutured tissues, proteomics on membrane microdomains, expression profile of genes involved in WH mechanisms, markers of fibroblast activation, ECM turnover at the sutured wound site, apoptosis and necrosis, stiffness and strength of the tissue at sutured wound site. The experiment design scheme is shown in Fig. 1.

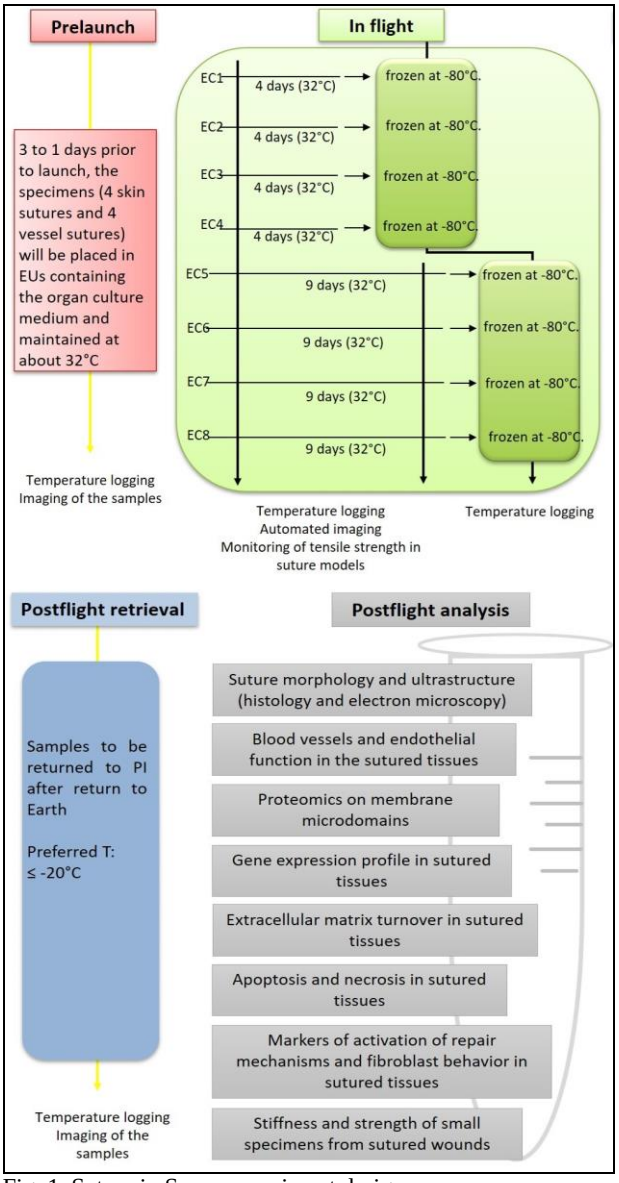

Fig. 1. Suture in Space experiment design

2.2 Ex vivo sutured wound model

For the preparation of the experiment it was necessary to face two challenging and closely related issues: i) ensure tissue survival and function throughout the experiment, lasting about 4 weeks; ii) define the requirements for hardware development. The two points were strictly interconnected because tissue survival strongly depends on collection protocols and culture conditions and hardware requirements had to be defined taking into account the optimal culture conditions.

Literature on tissue and organ cultures and results of our preliminary studies [12,13] suggested that both biochemical and mechanical factors are important in making the culture conditions more similar to the physiological ones. Mechanical factors play important roles in the regulation of both tissue homeostasis and repair mechanisms, therefore, culture conditions that model the physiological tensile strength in the tissue improves its survival.

Based on these premises, the activity for the model preparation focused on the following points:

1) improvement of biopsy collection procedures for restoring as soon as possible the tensile strength in the tissue and providing the necessary biochemical factors;
2) improvement of standard organ culture media by adding substances promoting tissue survival;
3) study of tissue mechanical properties in order to define the requirements of a device which would allow to restore the tensile strength in the tissue and monitor its changes;
4) study of oxygen consumption in tissue cultures to define the requirements for gas exchange in the culture chamber inside the EU.

As a result of the above studies and tests, a procedure for preparing and maintaining ex vivo sutured wound models was defined, as described below.

Human biopsies are collected by surgeons in operative rooms from patients undergoing plastic or cardiovascular surgery, under informed consent and Local Ethics Committee approval. The selected donors are not affected by infectious, chronic or oncologic diseases. These biopsies are taken from tissue specimens usually discarded after surgery and their use absolutely does not interfere with the patients, the administered therapies or the standard surgical procedures. Square skin biopsies (2 cm side) are excised by a scalpel while vein vessel biopsies (2 cm length, ~ 0,5 cm Ø) are cut by scissors. Immediately after excision the tissue specimens are dipped in Phosphate Buffered Saline (PBS) and then stitched to frames designed to stretch the tissue by applying a mechanical tension similar to the physiological one (see 2.4). During this procedure PBS is dripped onto the samples to keep them moist all the time. Finally, the frames with skin and blood vessel samples are placed in culture chambers filled with RPMI-based culture medium, modified by adding serelaxin (recombinant human H2 relaxin hormone, 60 ng/ml) and metal-nonoate Zn(PipNONO)Cl (28 ng/ml) and maintained at 4°C for 8-12 days (to simulate the transport from the site of sample collection to the launch site).

For experiments on WH, sutured wound models are prepared from the *ex vivo* tissue cultures (how it will be done at the launch site for the in-flight experiment. Little cuts (10 mm length, 2 mm depth) are performed on the skin samples by a scalpel in order to mimic a wound (Fig.2). The vessels are completely divided by scissors to perform an end-to-end vascular anastomosis. The skin wounds are sutured with interrupted stitch 3.0 non absorbable suture (Nylon) and vessels with continuous 6.0, 7.0, non absorbable suture

(Polypropylene). Then, the tensile strength in the sutured wound models is controlled and the models are placed in culture chambers filled with DMEM-based culture medium, modified by adding serelaxin (60 ng/ml) and the metal-nonoate Zn(PipNONO)Cl (28 ng/ml). The models are now ready to be used for a WH experiment. For the in-flight experiment, 8 sutured wound models will be prepared at the launch site: 4 skin sutures and 4 vessel sutures.

The applicability of the ex vivo sutured wound models described above to an experiment on the ISS has been tested simulating different experiment scenarios, in particular with regard to the timeline and temperature conditions: after wounding and suturing, the models have been cultured and monitored for 4-6 days at about 24°C (to simulate handover and upload on the ISS) and for 9-12 days at 32°C (to simulate the in-flight experiment). After many experiments and tests, including the hardware's Science Verification Test (SVT) and the operational Experiment Sequence Test (EST), samples have been frozen and stored at -80°C. Then, the survival of the tissues has been assessed by histology.

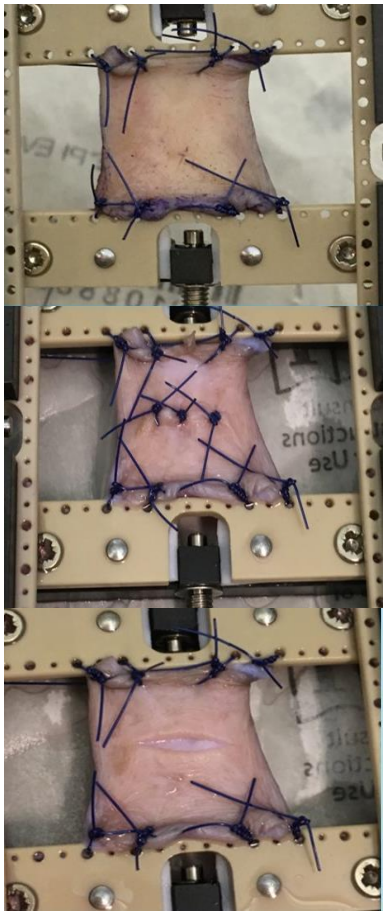

Fig.2. Sutured wound model (skin)

In order to define the requirements of the device for stretching the tissue samples and monitoring their tensile strength in the in-flight experiment, tissue mechanical properties (in particular, the tensile strength) have been studied using a prototype developed by Kayser Italia for the principal investigator's laboratory. The prototype consists of a metallic frame made of stainless steel, allowing the biopsies to be stitched on tissue support brackets made of biocompatible plastic, and stretched by tensioning screws. The tissue sample tension is measured and monitored over time by a commercial load cell and acquisition software, from Burster Srl (Fig.3)

Oxygen consumption in the tissue cultures has been studied in collaboration with Giuseppe Coppola's group (CNR Naples, Italy), who made available an oxygen optical sensor and a sealed measurement cell specifically developed for measurements of oxygen consumption in biological samples. Tests have been performed maintaining the tissue cultures in the sealed measurement chamber completely filled with medium (DMEM, about 25 ml), both at room temperature (22°C ± 2) and experiment temperature (32°C ± 2), humidity 53%. Oxygen consumption was monitored for minimum 24 h (Fig.3).

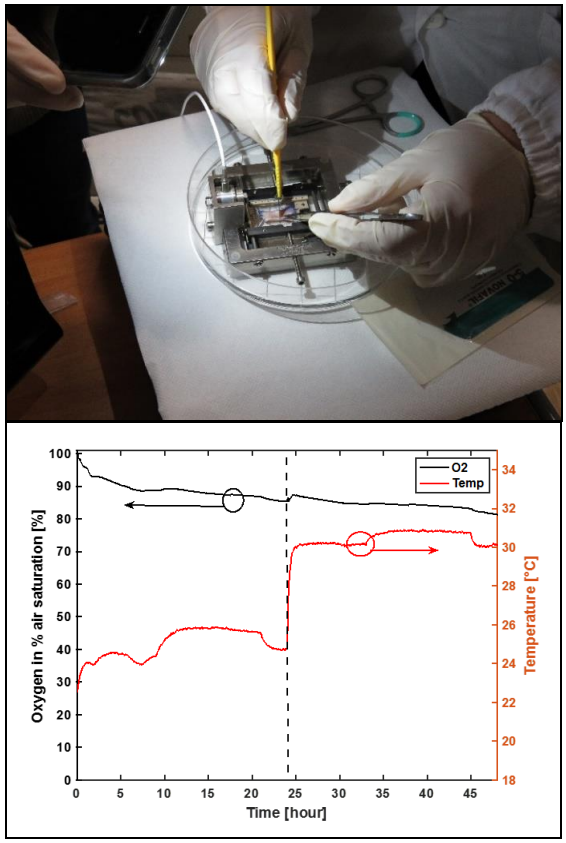

Fig.3. Tensile strength measurement in skin sample (upper); oxygen consumption monitoring in blood vessel culture at 24°C and 32°C (below).

2.3 Histology

At the end of experiments and tests, frozen tissues samples were quickly removed from the frame and processed according to the following method: samples were fixed in 4% paraformaldehyde in phosphate buffer (BioOptica Milano S.p.A., Milano, Italy) for 24 h and then dehydrated in graded ethanol and embedded in paraffin. Histological cross sections, 5 µm thick, were cut with a microtome (HistoCore MULTICUT, Leica, Milano, Italy).

Then, sections were stained with: i) hematoxylin and eosin (Bio OpticaMilano S.p.A., Milano, Italy) for conventional histological observation; ii) Picrosirius red (Sigma-Aldrich, St. Louis, MO, USA) for assessment of collagen fibres content; iii) aldehyde fuchsin (Acros Organics, Geel, Belgium) for assessment of elastic fibres content; iii) anti-Ki67 antiserum revealed by immunoperoxidase (Sigma-Aldrich, St. Louis, MO, USA) for proliferation activity; TRITC conjugated avidin (Sigma-Aldrich, St. Louis, MO, USA) for mast cells detection under fluorescent UV microscopy.

Digital photomicrographs were taken with a Nikon DS Fi2 digital camera and NIS Elements image acquisition software (NIS Software Ltd., Florence, Italy) and with a Visiscope Series 300 light microscope (VWR, Leuven, Belgium), interfaced with a VisiCam TC 10 Tablet Camera (VWR, Leuven, Belgium) by dedicated software.

2,4 Hardware development

The Suture in Space hardware has been developed by Kayser Italia. It is composed of a set of bioreactors allowing the culture and monitoring of *ex vivo* sutured wound models and the study of WH mechanisms. Each bioreactor is composed of a stainless steel tissue frame, conferring mechanical support to the skin or vein tissue, a culture chamber filled with culture medium, and a culture medium reservoir, containing the fresh medium allowing the culturing of the tissue sample for more than two weeks from the last integration step of the experiment in the hardware on ground until the end of the experiment on board the ISS. Fresh medium is added to the culture chamber via a peristaltic pump controlled by electronics. The body of the culture chamber and reservoir is made of a biocompatible plastic. Cabin air is allowed to reach the tissue sample via an application of a gas-permeable membrane to the culture chamber. All the hardware components in contact with the tissues and culture media have been selected in order to be biocompatible and autoclave steam sterilizable. The tissue sample, via a mechanical support, is connected to a load cell (Burster Srl) able to measure the tensile strength (in the range of 0-20N) in the sample during the experiment onboard the ISS. A dedicated acquisition electronics have been designed in order to allow the implementation of such device in the Suture in Space EHW. The Suture in Space electronics and microcontroller is located inside the AEC, and connectors allow the interface of the AEC electronics to the Biolab. The configuration allows the communication of the AEC to communicate with the Biolab to receive any command: e.g. pump activation start/stop, load cell acquisition etc..., and to send the status of the experiment. The sequence of operations will be managed by the scheduler of the Biolab.

4. Results and Discussion

The studies described in this paper have been conducted with the aim to prepare the Suture in Space experiment, which is focused on the study of WH in weightlessness and will be conducted aboard the ISS.

In this experiment, it was quite challenging to find culture conditions that ensure good preservation of the samples throughout the experiment (about 4 weeks) and that could be reproduced within the EHW.

Generally, when a biopsy is excised from the body, the tissue shrinks. Our preliminary studies, in agreement with other authors [12], demonstrated that tissue culture survival can be improved by stitching the biopsy to a frame for restoring approximately the initial size and, consequently, the physiological tensile strength. The values of tissue tensile strength reported in literature are very heterogeneous, because they depend on many factors [14]. Using a device to stretch the tissue until it reaches exactly the initial size and to measure the tensile strength (see 2.2 and 2.4), it was found that in the biopsies collected to prepare the sutured wound models, the tensile strength is < 10 N and depends on tissue type, body area from which the sample has been collected, donor age and gender. As expected, the tensile strength values changed after cutting, suturing and also during the experiment, due to tissue reaction to stretching, healing mechanisms and suture behavior. Based on the measurements described above, the requirements for monitoring the tensile strength throughout the in-flight experiment were defined as follows: measuring range: 0-20 N, sensitivity: ≤ 0.5 N, accuracy: 10% of the measured value.

Oxygen supply and diffusion into tissues are necessary for their function and survival. The oxygen partial pressure (pO_2) is a key component of the physiological state of an organ. It results from the balance between oxygen delivery and its consumption in a physiological condition. The different organs and tissues are characterized by their own and unique “tissue normoxia status”. Currently, *in vitro* and *ex vivo* experiments with cell and tissue cultures are usually performed in 19.95% O_2, but several studies suggest that this value is overestimated in comparison with physiological conditions, in which “normoxia” appears to be ≤ 11% O_2 in most tissues and organs [15].

Therefore, to define the oxygen requirement for tissue culturing inside the EHW, we directly measured oxygen consumption in skin and vein vessel cultures (see 2.2). As expected, oxygen consumption varied depending on tissue type and preservation conditions, time after collection, temperature and medium salinity. Based on the results of the measurements, oxygen consumption in the cultures resulted ≤ 0.5 µm/ml/h, for salinity ranging from 0 to 10 g/l.

Part of our work was devoted to find optimal composition of culture medium. Starting from media already used for organ and tissue cultures and described in literature, we found that they can be improved adding antioxidant, anti-inflammatory and proangiogenic molecules. So, we developed a new composition of the culture medium which improves viability and survival of tissue cultures in comparison with the media currently used. In both the culture media (RPMI- and DMEM-based), the addition of serelaxin (60 ng/ml) and metal-nonoates, in particular Zn(PipNONO)Cl (28 ng/ml), significantly improved tissue survival and preservation in long-term cultures.

Nitric Oxide (NO) released by nonoates (as Zn(PipNONO)Cl) in culture medium promotes endothelial cell survival, exerts anti-inflammatory, anti-thrombotic and anti-atherogenic properties [16,17]. The vasoprotective activities of nonoate-derived NO are direct, fast and prolonged since NO also induces intracellular signaling (i.e. the biosynthetic pathway leasing to H2S upregulation) leading to endothelial nitric oxide synthase (eNOS) activation and endothelial proper functioning [17,18]. The outcomes of tests carried out on *ex vivo* sutured wound models kept in culture for over 4 weeks further confirmed the effects reported in literature by other authors.

Serelaxin is the recombinant form, suitable for pharmacological use, of human H2 relaxin hormone, a member of the relaxin-like peptide family, which shares structural similarities with insulin but is endowed with completely different effects. Serelaxin acts by multiple molecular mechanisms, chiefly up-regulation of the cAMP, the nitric oxide/cGMP and the endothelin-B receptor pathways, by which it exerts prominent on the cardiovascular system, such as relaxation of the vascular wall to promote tissue perfusion, induction of VEGF expression to promote neo-angiogenesis, enhancement of matrix metalloproteinase (MMPs) expression to promote collagen breakdown and connective tissue remodeling [19,20]. Several experimental studies have found that serelaxin exerts a marked protective function against ischaemia-reperfusion injury, by reducing cellular oxidative damage, apoptosis and inflammation [21,22]. By similar mechanisms, serelaxin has shown to extend the lifespan of explanted organs (liver, lung) scheduled for transplantation [23]. On this background, we hypothesized that serelaxin could be used to improve the viability of the explanted skin and blood vessel biopsies during long-term ex-vivo experiments. The results obtained in the tests performed on the *ex vivo* sutured wound models demonstrated the validity of our hypothesis.

It is noteworthy that both metal-nonoates and serelaxin, acting sinergistically on endothelial function, not only improved the survival of blood vessel cultures, but also that of skin cultures.

Tissue viability and preservation of morphological structures in the *ex vivo* sutured wound models has been assessed by histological analysis. Skin and blood vessel samples have been cultured with the newly developed technique above described (see 2.2 and 2.4) and based on both mechanical and biochemical factors. At the end of tests simulating the scenario of the space experiment with regard to timeline and temperature profile (see 2.1 and 2.2), the samples have been frozen, then processed and analyzed.

In histological preparations from skin samples, hematoxylin eosin staining showed that morphological structures were well preserved. The epidermis showed well-maintained histological features, with no detachment of corneum layer and substantially conserved basal and suprabasal layers; the dermis was also well-preserved, with no signs of cell demise or staining abnormalities (Fig. 4). Ki67 immunostaining confirmed the presence of proliferating cells in the tissue (Fig. 5) Mast cells, revealed by staining with TRITC conjugated avidin, also appeared normal (Fig. 6). ECM architecture was substantially maintained: in particular, picrosirius red staining of collagen fibers showed a well preserved collagen network (Fig. 7).

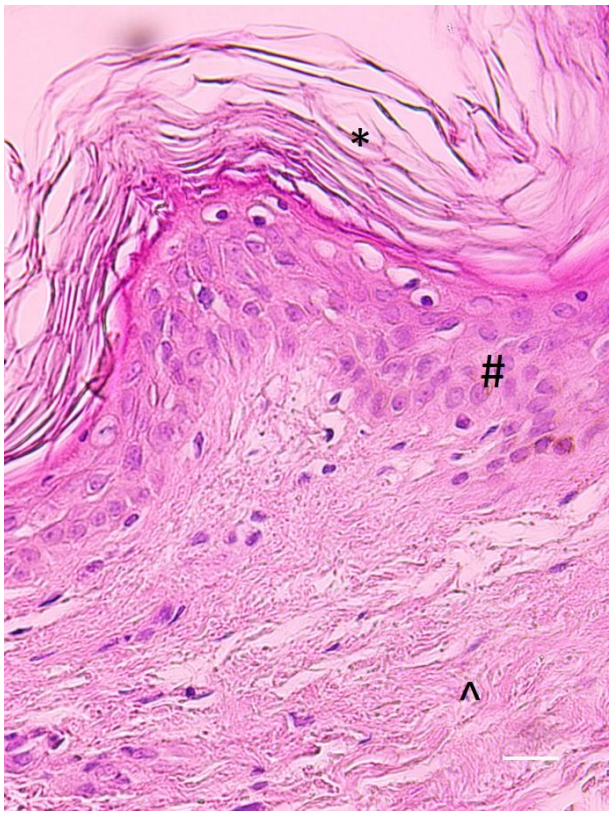

Fig.4. Skin sample - Hematoxylin-eosin staining. Bar 50 µm (x40). Good preservation of the various layers: epidermis with well maintained stratum corneum (*) and stratified squamous epithelial tissue (#) and dermis (^).

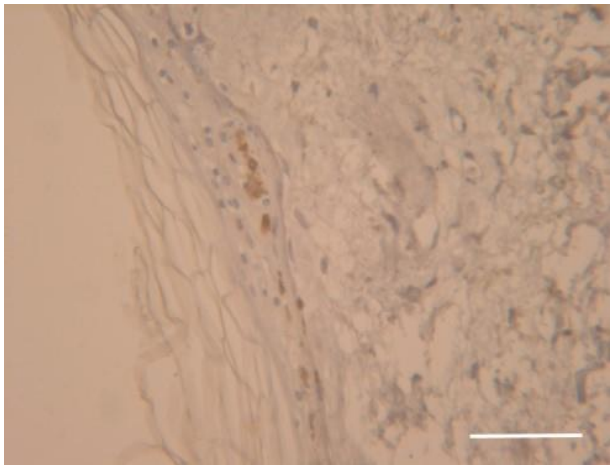

Fig.5. Skin sample - Ki67 immunostaining. Bar 50 µm (x25).

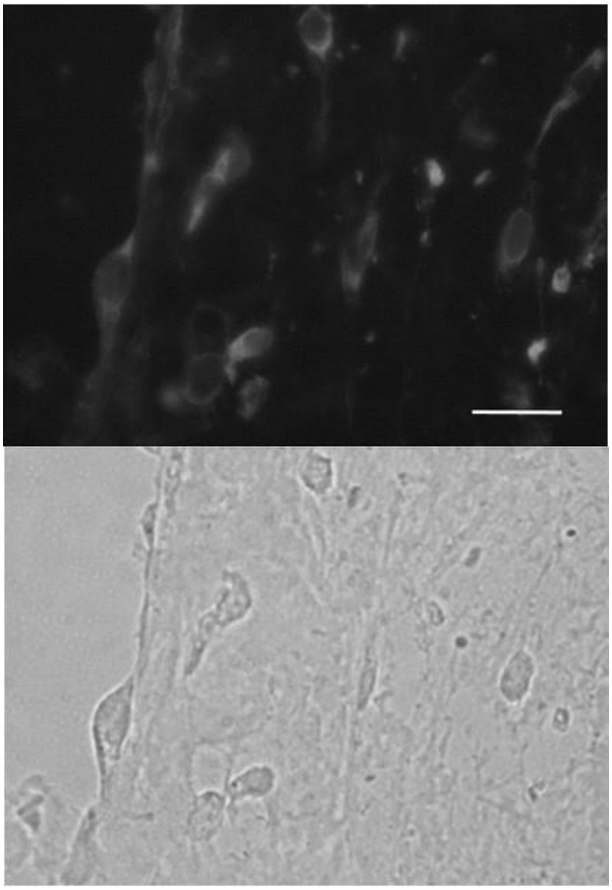

Fig.6. Skin sample - Mast cells stained with TRITC conjugated avidin (top) and relative phase contrast image (bottom). Bar 10 µm (x100).

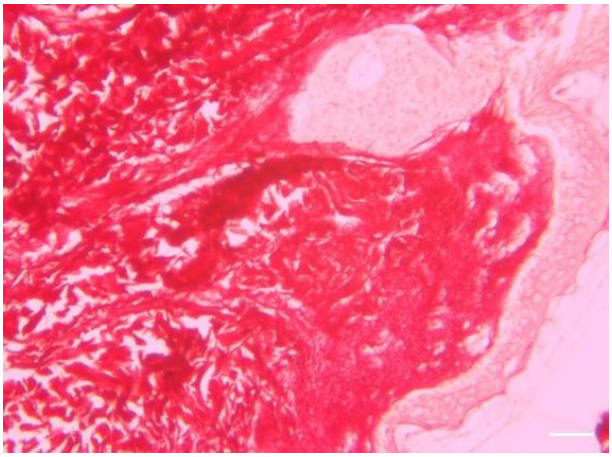

Fig.7. Skin sample - Collagen fibers stained with Picrosirius red. Bar 50 µm (x10)

Histological preparations of vein vessel samples stained with hematoxylin eosin showed good preservation of the three layers of the vessel wall: e.g. intima, media and adventitia (Fig. 8).

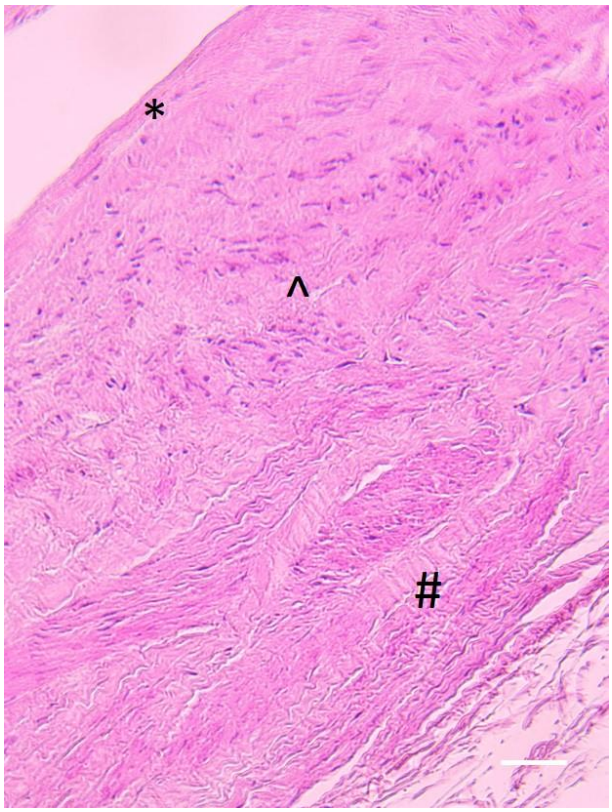

Fig.8. Vein vessel sample - Hematoxylin-eosin staining. Bar 25 µm (x20). Good preservation of morphological structures. The layers of the vessel wall can be observed: tunica intima (*), tunica media (^) and tunica adventitia (#).

A normal staining pattern was maintained. Picrosirius red staining and aldehyde fuchsin staining demonstrated preservation of the collagen and elastic fiber networks, respectively (Figs 9 and 10).

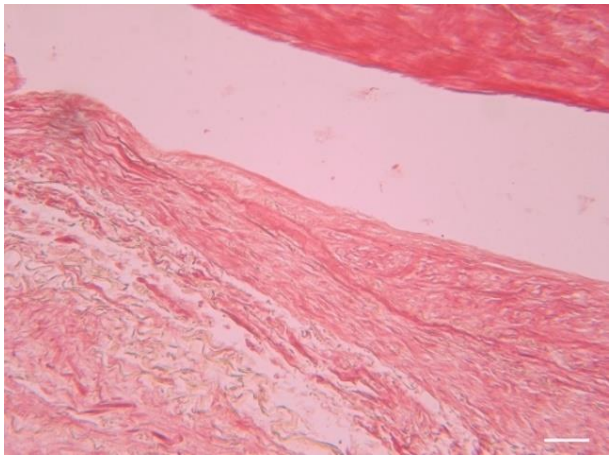
Fig.9. Vein vessel sample - Collagen fibers stained with Picrosirius red. Bar 50 µm (x10)

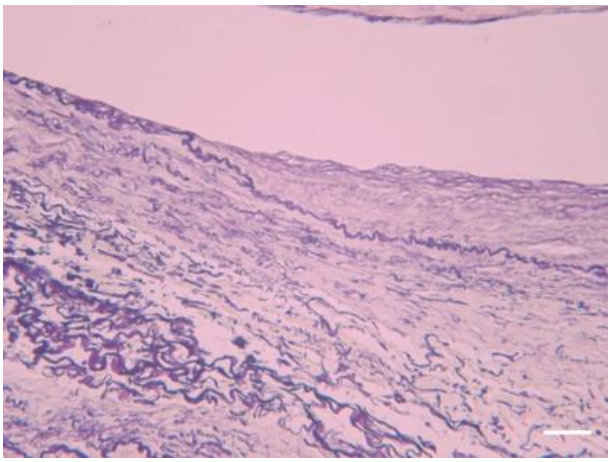
Fig.10. Vein vessel sample - Elastic fibers stained with aldehyde fuchsin. Bar 50 µm (x10)

In summary, the results obtained show that human skin and blood vessels, cultured under stretching and in presence of culture media modified by adding serelaxin and metal-nonoates can survive for over 4 weeks and, conceivably, retain the ability to trigger the mechanisms involved in tissue healing, such as proliferation and re-epithelialization (Fig. 11)

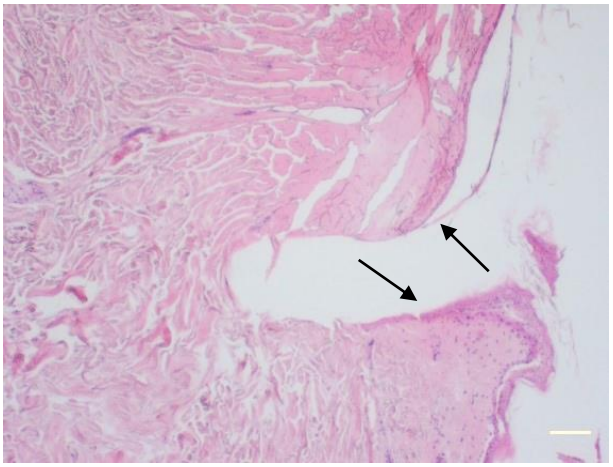
Fig.11. Skin sample - Hematoxylin-eosin staining. Bar 100 µm (x20). Arrows indicate the re-epithelization process.

These findings show that the culture technique we developed ensures the survival of the samples throughout the whole in-flight experiment. Moreover, the new culture technique can lead to benefits on Earth, in the fields of tissue/organ culturing and storage (tissue banks), tissue regeneration and engineering.

From the EHW perspective, all the functional tests performed during the development of the hardware, together with the SVT and EST, have shown that the hardware developed for the Suture in Space experiment is able to sustain the scientific protocol proposed by the Science Team. Science wise, the EHW allowed the preservation of the tissue samples over time (i.e. no

microbiological contamination occurred, tissue quality at the end of the tests were considered as nominal as the ones performed in common laboratory devices), and the acquisition of tensile strength data during the course of the experiment.

6. Conclusions

In conclusion, the activities for preparing the Suture in Space experiment led to obtain two outcomes with interesting application potential:

1) the development of new ex vivo sutured wound models which can be used in Space and on Earth to study: i) WH mechanisms and the role played by gravity and mechanical factors; ii) the effectiveness of suturing materials and techniques in restoring the tensile strength in the tissues and promoting scarless healing, iii) the effectiveness of new tools and countermeasures (drugs, dressings, scaffolds, devices, etc…) aimed at improving wound monitoring, management and healing.
2) The development of a new tissue culture technique based on the synergistic effect of mechanical and biochemical factors to model culture conditions closest to physiological ones. This tissue culture technique can be applied in Space and on Earth to: i) study the mechanical properties of tissues and tissue constructs; ii) improve and extend tissue/organ survival/lifetime throughout tissue/organ collection, preservation and transport. This application can be useful for regenerative medicine, tissue banks, organ and graft transplant.

Moreover, the culture technique, including the device for tissue stretching and the monitoring of tissue mechanical properties, which is currently aimed at skin and blood vessel culturing, can be modified and adapted to different soft tissues and tissue constructs.

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