reached in the intracellular- and blastocoel space within seconds after transfer to VS, regardless of step 1 duration. But with a short step 1, the cells will be vitrified while being severely shrunken. These simulations (and indeed experimental evidence we obtained in equine oocytes) suggest there may be an optimum balance of the risk of damage due to long exposure to VS and the (assumed) risk of vitrification of embryos in severely shrunken, condensed condition.

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**Conflict of Interest:** None to disclose.

## S129
## CRYOTHERAPY TEMPERATURE EFFECTS ON FUNCTIONAL AND ONCOLOGICAL OUTCOMES IN PROSTATE CANCER

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Cryotherapy, using a target temperature of -40°C, is an effective definitive treatment for organ-confined prostate cancer. We sought to determine whether a moderate minimum tumor temperature (below -40°C but above -76°C) is associated with improved quality of life, and/or an increased risk of disease recurrence relative to a very cold temperature (below -76°C).

An IRB-approved database was reviewed for patients who underwent primary cryotherapy for organ-confined prostate cancer from 2004 to 2017. Cohort characteristics were compared using descriptive statistical analysis. EPIC and IIEF quality of life questionnaire responses in the 4 years following treatment; and biochemical recurrence, post-treatment positive biopsy, progression to salvage treatment, metastasis, and overall survival truncated at 6 or 8 years post-treatment (median follow-up 30 [IQR: 33] months) were analyzed and compared using ANOVA, t-tests, Kaplan Meier and log-rank analyses. Patient cohorts were stratified based on whether their minimum tumor temperatures were colder (″very cold″) or less cold (″moderate-cold″) than -76°C, the median minimum tumor temperature for our cryotherapy patients as determined via chart review.

144 patients had moderate-cold minimum tumor temperatures, and 134 had very cold minimum tumor temperatures during cryotherapy procedures. EPIC questionnaire data were available for 52 patients in the very cold group and 64 patients in the moderate-cold group in the 4 years following treatment. The groups with available questionnaires did not differ in age ($p=0.66$), preoperative PSA ($p=0.08$), or preoperative Gleason scores ($p=0.13$). The groups did not differ in patient-reported urinary function ($p=0.77$) or bowel habits ($p=0.15$). Moderate-cold minimum tumor temperature was associated with superior (post-operative year 2, $p=0.03$) and more rapid improvement in sexual function scores relative to the very cold cohort. Moderate-cold versus very cold minimum tumor temperature showed no difference in biochemical recurrence ($p=0.60$) post-treatment positive biopsy ($p=0.95$), progression to salvage treatment ($p=0.40$), metastasis ($p=0.47$), or overall survival ($p=0.06$).

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**Conflict of Interest:** None to disclose

## S130
## CRYOABLATION IN THE TREATMENT OF LUNG CANCER

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Lung cancer is the most commonly diagnosed cancer in the United States and Europe and it is a major cause of cancer death. Surgical resection, when possible, offers the best chance of healing of NSCLC in selected patients and in early stage. In patients not candidates for surgery, chemotherapy and radiotherapy are mainly palliative. Cryoablation is a minimally invasive technique, highly innovative, which has only recently been used in the treatment of primary and secondary lung tumors. Cell death is obtained as a result of rapid freezing followed by slow thawing that causes necrosis of the target tissue. Cryoablation can be proposed with radical intent (curative) in cases of disease limited to the lung; individual tumors no larger than 5 cm or up to 5 multiple tumors confined to no larger than 3 cm each one. The advantages of cryoablation are due to very precise control of the treated area (display of the iceball) sparing the surrounding healthy tissues. The major risks and complications of pulmonary cryoablation are those deriving from interventional treatment such as: local hematomas, pneumothorax, pulmonary bleeding caused by wrong placement of the cryoprobes and infections.

**Source of Funding:** Not applicable

**Conflict of Interest:** None to declare

## S131
## AN EXPERIMENTAL AND NUMERICAL APPROACH FOR NODULAR SKIN TUMOUR ABLATION USING CRYOTHERAPY

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Liquid nitrogen spray cooling has been performed to quantify the necrotic zone in the nodular gel phantom. The tissue-mimicking gel is assigned with two different configurations of nodular gel phantom, i.e., first: 4.5 mm depth and 5.5 mm radius; second: 4 mm depth and radius. The spray cooling is carried out using 0.8 mm nozzle diameter, 27 mm spraying distance and 120 s freezing for the experimental study. The multi-block non-orthogonal grid is used for the mathematical model and enthalpy equation is solved with finite volume approach. The variation of temperature and ice front in the nodular gel phantom are evaluated experimentally for both the nodular configurations and corroborated with the numerical study. The lethal temperature (-50°C), measured with the thermocouple is obtained up to 4.5 mm and 4 mm depth in the first and second configuration of nodular gel respectively. The final ice front measured using Image J software in the axial and radial direction for the first configuration is 9.1 mm and 10. 1 mm respectively while for second configuration it is 9.7 mm and 10.4 mm respectively. The ablation volume characterised by -50°C and -25°C is quantified numerically for the application of a malignant and benign tumour respectively. The final ablation volume enclosed by -50°C is 67% and 76% lesser than final ice volume obtained by first and second configuration respectively while for -25°C it is 51% and 61% respectively. The cryogen spray cooling with 0.8 mm nozzle diameter with spraying distance of 27 mm can be suitable for benign skin tumour with both the configurations unlike the second configuration for malignant skin tumour.

**Source of Funding:** Not applicable

**Conflict of Interest:** None to disclose

## S132
## WITHDRAWN

## S133
## AN ALLOGENEIC BIOSCAFFOLD FOR THE STORAGE OF HUMAN MESENCHYMAL STEM CELLS.

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MSCs derived from synovial fluid can provide high chondrogenic and

cartilage regeneration, emerging as a new alternative stragegy to treat knee ostoearthitis. We have developed an allogeneic and biomimetic bioscaffold composed of Platelet Rich Plasma and synovial fluid that preserve and mimics the natural environment of MSCs isolated from knee. However, cryopreservation of knee-isolated MSCs embedded within the aforementioned biomimetic scaffold to create a reserve of young autologous embedded knee MSCs for future clinical applications remained unsolved. Thus, we tested several cryoprotectant solutions combining dimethyl sulfoxide ($Me_2SO$) and sucrose, quantifying MSCs viability and functionality after thawing. MSCs embedded in bioscaffolds cryopreserved with $Me_2SO$ 10% or the combination of $Me_2SO$ 10% and Sucrose 0,2 M displayed the best cell viabilities and functionality after thawing, allowing their future clinical use in patients with cartilage defects.

**Source of Funding:** Not applicable

**Conflict of Interest:** None to disclose

## S134

## TOWARDS LARGE-SCALE CRYOPRESERVATION: STERILE VITRIFICATION OF ADHERENT HUMAN INDUCED PLURIPOTENT STEM CELLS AND THEIR NEURAL DERIVATES

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Cryopreservation is still the only possibility to store viable cells for long periods. In general, conventional slow freezing methods are efficient enough when it comes to banking of single cells in suspension for subsequent expansion (e.g. human induced pluripotent stem cells, hiPSCs). However, considering adherent cells or multicellular systems that are increasingly relevant for biomedical research and application, slow freezing shows major limitations. (1) Usually the adherent cells have to be enzymatically or mechanically dissociated to single cells or small aggregates prior to freezing, (2) crystallization-induced damaging mechanisms additionally disrupt cadherin- and integrin-mediated cellular contacts, and especially for hiPSC, (3) the recovered viable cell numbers is dramatically reduced compared to the control. Vitrification provides the possibility to overcome these limitations, but requires skilled handling especially regarding sterile procedures, imply small sample sizes and therefore is considered as unsuitable for bulk storage. To launch vitrification for large cell numbers and thus enabling ready-to-use cryopreserved adherent cell systems, we introduce a sophisticated multi-usage cell culture disposable covering comprehensive cell-based workflows from cultivation/differentiation to sterile vitrification. We validated this disposable by a comparative multi-centre study, examining adherent vitrification in the disposable and of suspension-based conventional slow freezing of six hiPSC lines and the accordant hiPSC-derived neural progenitor cells (NPCs). Viability, cell number, immuno staining and FACS as well as gene expression and raster electron microscopic analysis were performed as post thaw quality controls after one day and four days, respectively. Our data shows superior performance of vitrification over slow freezing of both cell systems. Higher numbers of viable cells and metabolic activity could be detected, while the functionality maintained. Together with the option to parallelize the disposable in multi-well formats, vitrification is applicable for large-scale cryopreservation of adherent multi-cellular systems and enables ready-to-use formats for a variety of biomedical purposes.

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**Conflict of Interest:** None to declare

## S135

## ADVANCES IN CRYOPRESERVATION OF ALGINATE-ENCAPSULATED STEM CELLS AND ANALYSIS OF CRYOPRESERVATION OUTCOME

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Cryopreservation of clinically relevant cells and biologics encapsulated in alginate hydrogels is an efficient methodology to preserve their functionality for further application in regenerative medicine and transplantation. The main advantages of alginate encapsulation for cryopreservation include possibility for process scaling up, encapsulation of required cell numbers within viscous environment, which in turn serves as a reservoir for cryoprotective agents, protects cells from mechanical and osmotic stress as well as provides controlled membrane permeability. In this work, we report on our success in cryopreservation of multipotent stromal cells within different alginate hydrogel formulations (solid and coaxial beads of different sizes), analysis of cell viability using the designed μVision software allowing for spatial reconstruction of cryomicroscopic images as well as ice recrystallization using μ Crystal software. The developed approach for cell encapsulation in coaxial alginate beads with varied membrane thickness provides effective formation of tissue-like 3D structures within the core. This approach could serve as a model for cryopreservation of self-assembled structures with cell-cell contacts. For solid alginate beads, optimization of cryopreservation parameters yielded intact alginate hydrogels after thawing according to cryomicroscopic data. High cell viability and recultivation efficiency 24h after thawing (viability 83% by Calcein-AM/EthD-1 staining and 70% by recultivation efficiency) have been achieved. In addition, optimal cryopreservation protocol has been successfully validated for freezing of cell structures within coaxial alginate beads, whereas μCrystal software proved to be efficient for analysis of cryomicroscopic images of ice recrystallization. Taken together, our work provides a comprehensive overview of the main results achieved by the group on cryopreservation of stem cells within alginate solid (microbeads) and coaxial hydrogels.

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**Conflict of Interest:** None to disclose.

## S136

## ME2SO- AND SERUM-FREE CRYOPRESERVATION OF MESENCHYMAL STROMAL CELLS USING ELECTROPORATION OF SUGARS

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Cryopreservation is the universal technology enabling continuous availability of cell aliquots to meet regenerative medicine demands. However, safety concerns over Me2SO-induced side effects and immunogenicity of animal serum (main components of standard freezing media), support their replacement with non-toxic substances. Due to multiple cryoprotective properties, selected disaccharides, such as sucrose and trehalose, are widely used as additives to various freezing solutions. Conceptually, combined introduction of sugars into cryopreservation media and their pre-freeze loading into cells serves as a novel alternative to conventional cryopreservation workflow. Among diverse techniques for sugar loading (e.g. fluid-phase endocytosis, genetically engineered proteins or nanoparticle-mediated delivery) electroporation is a preferred method in cryopreservation owing to its high-performance speed, safety and accuracy. In this study, we investigated the effect of electroporation-assisted