SIMULATIONS OF OSMOTIC EVENTS IN VITRIFICATION OF EQUINE OOCYTES AND PORCINE EMBRYOS

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deficiencies, here we apply, inform, and test agent-based cell models of cryoprotectant equilibration in secondary preantral follicles. Our focus is on the equilibration of cryoprotective agents (CPAs), a critical part of all successful cryopreservation protocols. In this study, we first derive a model and develop computer code describing CPA equilibration in a 2D tissue model of a secondary stage ovarian follicle. This model accounts for intercellular connections using a modified spring force potential equation, mass transport using a combination of a prebuilt diffusion solver and two-parameter volume equation, concentration based CPA toxicity using a cost function and additional cell mechanics such as motility and cell-membrane rigidity. To do this, we use a highly customizable cell mechanics and interstitial diffusion simulator based on an agent-based model of cells in nonhuman primate ovarian follicles. We inform this model with literature values and explore the defining features of this model numerically. Our simulations show a marked difference in volume change between the granulosa layer and oocyte that may suggest mechanistic explanations for cryopreservation induced damage.

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126
TOWARDS NEW PROTOCOLS OF OVARIAN TISSUE CRYOPRESERVATION ASSISSTED BY X-RAY COMPUTED TOMOGRAPHY

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Ovarian tissue cryopreservation is in most cases the only option for female cancer patients to preserve their fertility, especially in the case of prepubertal girls. So far, the cryopreservation of ovarian tissue has been performed mostly by the conventional *slow freezing* method. Even though there are more than 100 live births from transplanted frozen-thawed ovarian tissue, there is evidence of follicle population damage associated with this cryopreservation procedure. The different characteristics between cells suspensions (traditionally cryopreserved by *slow freezing*) and tissues might be the main issue of this follicle loss: tissues are composed of different cells which are densely packed and besides, they have a bigger volume. The purpose of this work was to develop an alternative protocol of ovarian tissue cryopreservation by a *stepped vitrification*, consisting of an increasing in cryoprotectant concentration while decreasing the temperature. This approach aims to reduce cryoprotectant toxicity and avoid permeability problems. CT measurements were performed after the cooling process (below -140 ^{o}C) in order to evaluate the cryoprotectant permeation in the tissues, and after tissues rewarming, to evaluate the cryoprotectant washing process. For these experiments, we used bovine ovarian tissue from one-year old animals. Histological analysis were also performed to evaluate the morphological state of the tissues. Bovine ovarian tissues cryopreserved by *slow freezing* were also compared with ovarian tissues vitrified with the presented method. CT images showed that the equilibration of tissues with the cryoprotectant solution was not complete in most samples of the new vitrification protocol developed, although an equilibration up to 80% of the desired concentration was achievable. Nevertheless, histology results revealed no significant differences between tissues from both protocols. Anyways, X-ray CT technology is proved to provide a very relevant information to adjust the parameters necessary for achieving a successful vitrification method.

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S127
BIOMECHANICAL MODEL OF CRYOPROTECTANT TRANSPORT IN TISSUES WITH HIGH CELL DENSITY

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Cytotoxicity from exposure to high cryoprotectant (CPA) concentrations is one of the major barriers to the development of effective tissue cryopreservation procedures. In previous work, our group developed a mathematical approach to minimize CPA toxicity, and applied it to isolated cells and cultured cell monolayers. Our recent efforts have focused on extending this toxicity minimization approach to larger biological specimens. In particular, we have focused on developing a model of CPA transport in tissues that accounts key biophysical phenomena, including mechanical strain and fixed charges in the extracellular matrix. While most previous modeling efforts are based on Fick's law and do not account for tissue size changes or fixed charges, recently a model of transport in articular cartilage was published that accounts for both of these phenomena. However, the published model neglects the effects of cells on interstitial transport. While this is a reasonable assumption for cartilage, most other tissues have much higher cell density. Thus, we have extended this model to account for the inherent coupling between interstitial transport and cell membrane transport. Model predictions reveal two key effects that are expected to affect design of tissue cryopreservation procedures. First, the presence of cells slows down delivery of CPA into the tissue interior. This is because cells act as a sink for CPA. Second, accounting for the presence of cells within the tissue reduces the magnitude of predicted cell volume changes. Physically this can be explained in terms of the interplay between cell size and fixed charge concentration. For instance, cell swelling concentrates fixed charges, which opposes further swelling. The model we present here can be used for tissues with a broad range of cell densities and will serve as the basis for future efforts to mathematically minimize CPA toxicity.

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S128
SIMULATIONS OF OSMOTIC EVENTS IN VITRIFICATION OF EQUINE OOCYTES AND PORCINE EMBRYOS

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High concentrations of cryoprotective agents (CPAs) used for vitrification of equine oocytes and porcine embryos are believed to negatively affect developmental competence. Here, osmotic events were simulated using 2P formalism (Kleinhans, Cryobiology 37, 271–289) to predict effects of reduced duration of CPA exposure and effects of use of non-permeating CPAs. Varying concentrations of permeating CPAs (ethane-1,2-diol and Me_2SO) were used in the first step (equilibration solution, ES) and second step (vitrification solution, VS). In VS, varying concentrations of additional non-permeating solute (sucrose) were used. The duration of the first step (ES) was varied between 0.5-10 min, while exposure to VS was 0.5-1 min. Values for Lp and Ps were based on published values for oocytes and embryos respectively.

Typical 'volume excursions' are seen, in which oocytes and embryos rapidly (<0.5 min) shrink in ES due to efflux of water, then start re-swelling due to combined influx of CPA and water. After transfer to VS, the oocytes/embryos shrink a second time, concentrating intracellular solutes including CPA taken up in step 1. In line with that, the simulations show that the allegedly advantageous reduction of the intracellular concentration of permeating CPAs by non-permeating sucrose is only very small. Further, although with a short (0.5 min) step 1 (ES), much less permeating CPA is taken up, yet osmolality equal to that of VS (≈ 'vitrifiability') is

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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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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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.

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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