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Final Report on Cryopreservation of Biological Tissues

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Abstract

This report summarizes the experimental and anayltical work that was done in support of Sandia National Laboratories. This work investigated the feasibility of obtaining validation data to support the development of a complex computer code that simulates the thermal and chemical response of living tissue to a freezing transient.

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This is to report on the work that we did on the contract from Sandia on the project entitled "Cryopreservation of Biological Tissues," with the performance period May 24 2000, to September 15 2000. Although the time was short, I believe we made significant progress towards addressing our research objectives and developing a long-term collaboration on modeling and experimental studies in the area of tissue engineering.

The specific tasks of the contractor (Georgia Tech) and the work accomplished in each are as follows.

1. Determine the type of data which are (i) experimentally feasible and (ii) suitable for model validation.

In collaboration with our technical contact at Sandia (Dr. Ronald Dykhuizen), we identified that the appropriate type of data to be collected include (i) the size of cells and (ii) the concentration of cryoprotective agent (CPA) as a function of radial position in spherical encapsulated cell constructs and following freezing/thawing procedures.

2. Perform a literature search to identify similar experiments.

We performed a thorough literature search, which yielded no publications with experiments of the same type. However, we did identify articles describing other useful studies, including the freezing of hepatocytes in different types of extracellular matrix.

The following article discusses the cryopreservation of alginate-entrapped cells. It indicates that alginate doesn't have many of the problems encountered by others when using collagen as an extracellular matrix.:

Guyomard, Claire, Rialland, Laure, Fremond, Benjamin, Chesne, Christophe, Guillouzo, Andre, "Influence of Alginate Gel Entrapment and Cryopreservation on Survival and Xenobiotic Metabolism Capacity of Rat Hepatocytes." Toxicology and Applied Pharmacology, 141: 349-356 (1996)

The following three articles discuss some of the problems caused by collagen:

Cattral, Mark S., Lakey, Jonathan R.T., Warnock, Garth L., Kneteman, Norman M., Rajotte, Ray V., "Effect of Cryopreservation on the Survival and Function of Murine Islet Isografts and Allografts." Cell Transplantation, Vol. 7 No. 4: 3373-379 (1998)

Pajot-Augy, Edith, Prost, Rene, Axelos, Monque A.V., "Cryosolvents Effects on Low Temperature Gel Structure of Denatured Collagen." Cryobiology, 28: 335-346 (1991)

Watts, P., Grant, M.H., "Cryopreservation of rat hepatocyte monolayer cultures." Human & Experimental Toxicology, 15: 30-37 (1996)

3. Develop a protocol for experiments, most likely using as a model system cells entrapped in spherical alginate beads.

To address this, we began by verifying that an appropriate model system would be cells entrapped in spherical alginate beads and by identifying the appropriate experimental techniques for collecting the data identified in paragraph 1 above. We confirmed that calcium alginate gels can withstand freezing down to -20C, -70C, or -196C (boiling point of liquid nitrogen), followed by thawing to room temperature, without collapsing. It should be noted that, although no structural changes of the gels could be observed microscopically when freezing occurred to -20C or -70C, such changes were noticed when beads were frozen to -196C and then thawed to room temperature. In no case, however, did the gels collapse.

To measure cell size distribution in beads, we decided to use confocal microscopy. To measure the distribution of CPA, we decided it would be best to section frozen beads with a cryotome, then thaw the sections and assay the CPA amount in each. An undergraduate research assistant (Mr. Sunny Singh) working on this project during the contract period became familiar with confocal microscopy, however, technical difficulties with our cryosectioning apparatus prevented him from developing sectioning protocols for the system at hand.

4. Perform experiments in which beads will be immersed in a solution at low temperature containing a cryoprotective agent and, following freezing, data will be collected.

We did not manage to complete these experiments during the contract performance period for the following reasons: (i) these experiments are much more involved than initially anticipated; (ii) we had technical difficulties with the cryosectioning apparatus during the summer. Nonetheless, the undergraduate research assistant became familiar with the techniques needed for these experiments, including cell culturing, cell encapsulation, freezing/thawing processes, cell counting and viability measurement, and confocal microscopy, and established most of the protocols needed for the proposed experiments. We are committed to continue this line of research, which we believe is of profound significance for ensuring the off-the-shelf availability of tissue engineered products. Thus, Mr. Singh and Dr. Sambanis will work on these experiments beyond the end of the Sandia contract period with support from the Georgia Tech/Emory Center for the Engineering of Living Tissues (GTEC). In addition, we plan to hire a graduate student using GTEC funds to work on this project.

5. Provide Sandia with experimental details that will allow simulation of the experiments at Sandia.

Please see paragraph 4 above for this. These experiments are in progress and data will be provided to Sandia as the experiments are performed.

6. Collaborate with Sandia to identify improvements to the model that will allow better agreement between experiments and predictions.

In spite of the experimental delays described in paragraphs 4 and 5 above, Drs. Dykhuizen and Sambanis developed a realistic model that incorporates a number of changes and improvements relative to initial model version. A paper describing this modeling effort was recently submitted for publication in Cryobiology.

In summary, we believe that we accomplished a significant amount of work during the performance period of this contract, and that we have set the stage for a long-term research effort in the area of cryopreservation of engineered tissues and of modeling tissue engineered substitutes.

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