A new method for uniformly cooling engineered tissues

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Aiming at the uniform cooling of the 3-D cultured tissues, we proposed in this study a new cooling method by perfusing low temperature fluid through the micro-pipes scaffold pre-configured inside the tissues. To illustrate the benefit of the new method and to test its advantages over the conventional surface cooling, theoretical modeling on the heat transfer process of the tissues subject to uniform spatial cooling was presented. Further, preliminary experiments were performed to test the practical output of the uniform cooling.

INTRODUCTION

In tissue engineering, cultured tissue must be preserved properly to ensure its viability and availability when needed in clinics. Cryopreservation has been one of the best choices to meet the request for long-term preservation. But still this approach is facing technological challenge of the unavoidable damage of the tissue when subject to freezing and thawing process [1]. During the process of decreasing temperature, the method of surface cooling is often adopted [2]. Therefore the cultured tissue is cooled from outside to inside, which will induce a non-uniform temperature distribution due to the large heat capacity of the cultured tissue and low thermal conductivity. And a relatively large temperature gradient will be produced, which will result in strong thermal stress and thus damage the tissues. To alleviate such adverse effect, we proposed in this study a new cooling method by perfusing low temperature fluid (gas or liquid, ethanol for example) through the micro-pipes pre-configured inside the tissues to realize uniform cooling. To illustrate the advantages of the new method over the commonly applied surface cooling, theoretical modeling on the heat transfer process was presented. Preliminary experiments were also carried out, and promising results were reported and discussed.

METHOD DESCRIPTION

This uniform cooling method is borrowing the idea from the concept of high heat transfer efficiency of blood vessel networks. As is well known in natural biological tissues, the spatially distributed vascular structure plays an important role in nutrition transport as well as heat transfer. This feature is of great importance and has been applied for resolving the problem of nutrition transport in tissue engineering [3,4]. There, to transport efficient nutrition substances and oxygen to the internal of the cultured 3-D tissues, special designed micro fibers are deliberately buried through the tissue scaffold, which serves as the flow pipes and bioreactors during the process of culture. The method was proved to be promising in solving the problem of nutrition supply in tissue culture. In analogy to this strategy, the spatially distributed pipes with running cooling fluid inside, similar to the blood vessel network, may also provide a new method of uniformly cooling the cultured tissue and thus realize its successful cryopreservation.

For the process of implementing this uniform cooling strategy, two main steps should be contained as follows. First, the material, amount and dimension of pipes should be strictly selected according to the specific needs of the tissues to be cultured and cryopreserved, then the pipes are spatially constructed and buried inside the tissue scaffold before culture. After days of successful culture, the pipes are spatially buried into the tissue. Note that the pipes will serve as the flow channels in the cooling stage, thus the

material of the pipes to be selected and the spatial construction should be optimized to fulfill the request of freezing tolerance and efficient heat transfer respectively. For the second step, the cultured tissue to be cryopreserved is cooled by immersing it into low temperature fluid (gas or liquid), at the same time, the fluid with the same temperature flows though the pipes to achieve a uniform cooling of the whole tissue. During the process of cooling down, the tissue temperature decreasing rate should be precisely controlled by changing the fluid temperature so that the minimum freezing injury can be obtained. Furthermore, different kinds of fluids can be used one after another to ensure the efficient heat transfer through the pipes at different temperatures. When the pre-designed temperature is reached, the fluid is removed from the pipes and then the tissue is preserved at the pre-designed temperature. Similar to the freezing process, the tissue can be thawed by perfusing warm fluids through the pipes.

COMPUTATIONAL SIMULATION

In order to compare the uniform cooling method with the commonly used surface cooling method, the mathematical model of heat transfer of the tissue is made and computational simulations based on it were carried out. Because the mathematical model is very similar to the bioheat transfer model in our previous work, the detailed description is not repeated here for brevity (see [5, 6] for reference). The tissue domain is prescribed in a rectangular geometry with $20\times50\times100mm^3$ in the x, y and z directions respectively. Four pipes is fixed in a proportional spacing manner at the horizontal plane throughout the tissue and the vessel diameter is set as 1 mm. Ethanol is adopted as the experimental fluid whose flow velocity is set as 10 cm/s and temperature -100 ℃. The results of the computational simulation are presented as follows.
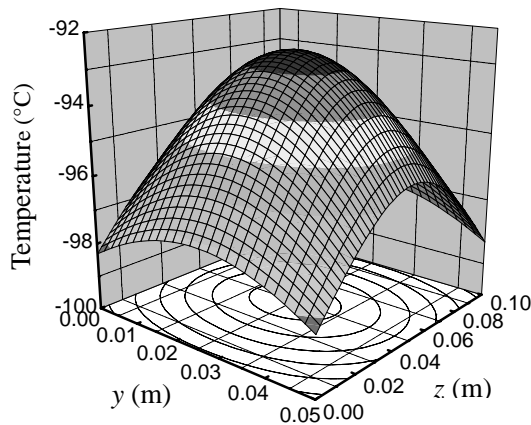

Figure 1 Temperature distribution at cross-section x=0.01m at t=960s for surface cooling method
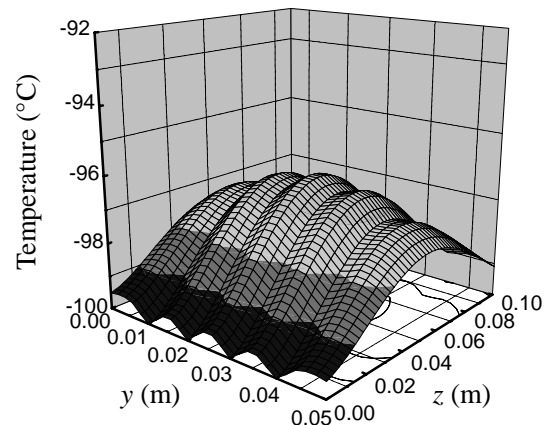
Figure 2 Temperature distribution at cross-section x=0.01m at t=960s for uniform cooling method

Figure 1 demonstrates the temperature distribution at the horizontal plane of the tissue by using the surface cooling method. At the surface of the tissue temperature drops dramatically due to large heat transfer coefficient of convection cooling. But inside the tissue, as a consequence of the large heat capacity of the tissue and relatively low thermal conductivity, large temperature gradient exists during the process of cooling. As shown in Figure 1, even after 960 s of surface cooling, the temperature difference between the center point and the edge point is 5 ℃. By contrast, the tissue subject to uniform cooling method has a rather uniform temperature distribution (see Figure 2) inside which the maximum temperature difference is about 2.5 ℃. The results indicate that by introducing the strategy of perfusing low temperature fluid through the tissue, heat transfer inside the tissue could be significantly enhanced due to the large heat transfer coefficient of convection. Thus smaller temperature gradient can be achieved, which is very beneficial for the successful tissue cryopreservation.

Figure 3 gives the temperature history of the center point of the tissue by using different cooling method. It can be seen from Figure 3 that compared to that of surface cooling method, a rather rapid cooling rate can be obtained by using this uniform cooling method, and the phase change region is much diminished in the curve. That is to say, the tissue with uniform cooling strategy experienced a shortened

stage of phase transition region, which may reduce the strong freezing injury of the tissue during the phase change process. It also can be concluded from the figure that to reach a pre-designed temperature, less time is needed by using uniform cooling method. In other words, the uniform cooling strategy has a shorter respond time.
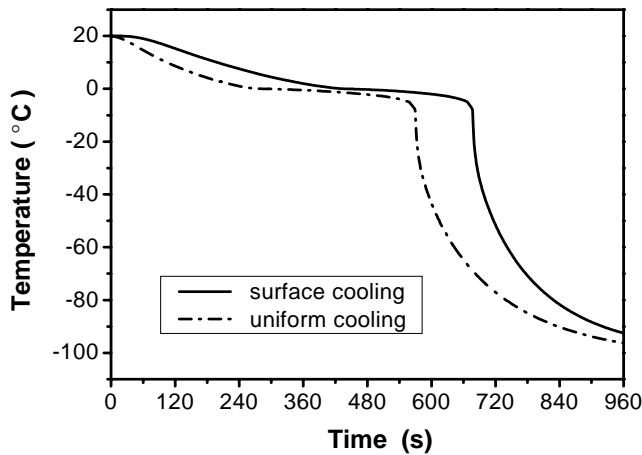

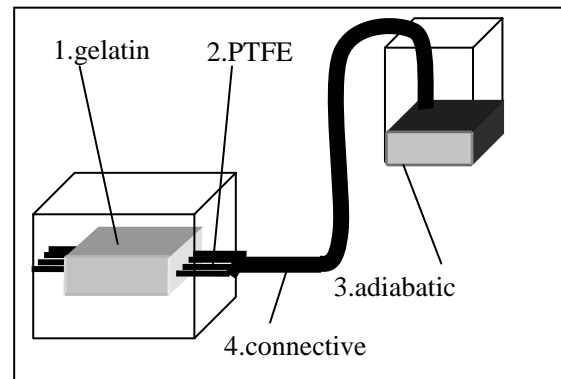Figure 3 Temperature history at the center point x=0.01m, y=0.025m, z=0.05m

Figure 4 Schematic illustration of the setup for uniform cooling

QUALITATIVE EXPERIMENT

To compare the practical effect of the new method and the commonly used surface cooling method for cooling cultured tissues, two qualitative experiments were designed and carried out. The setup is composed of three main parts (see Figure 4): a gelatin block 1 (14mm×40mm×80mm) with four parallelly aligned polytetrafluoroethylene (PTFE) pipes 2 (1mm for diameter) buried in, an adiabatic container 3, and a plastic pipe 4 to connect these two parts. The block A (see Figure 5) was placed in cold ethanol which is pre-cooled to -65 ℃ for 15 minutes. Its upper surface was not immersed in ethanol for the purpose of convenient observation. Note that there was no ethanol flowing in the pipes in Block A. For the block B (see Figure 6), the former step was repeated, at the same time, the cold ethanol (-65 ℃) was added to the adiabatic container and flew through the plastic pipe and PTFE pipes. The ethanol flux through each PTFE pipe was controlled at the velocity of about 4.5 ml/s.
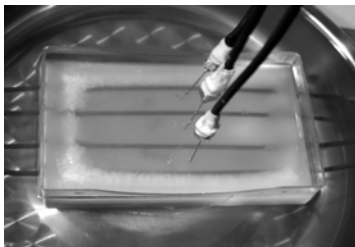

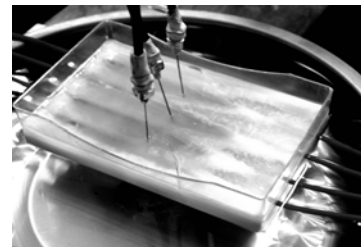Figure 5 Gelatin block A by using surface cooling with no ethanol in pipes at 200s

Figure 6 Gelatin block B by using uniform cooling with ethanol flowing through pipes at 200s

As shown in Figure 5 and Figure 6, the difference of cooling effect by these two methods is evident. For block A, ice is only formed around the gelatin, and the center of the gelatin remains unfrozen. But for block B, ice is not only formed around the gelatin, but also surrounding the PTFE pipes. Further, three thermal couples were placed in the gelatin to examine the temperature history of the two method. Take the center point for example, as shown in Figure 5, the differences in temperature transient resulted by two cooling strategy were also large. Compared to that of surface cooling method, a rather rapid cooling rate
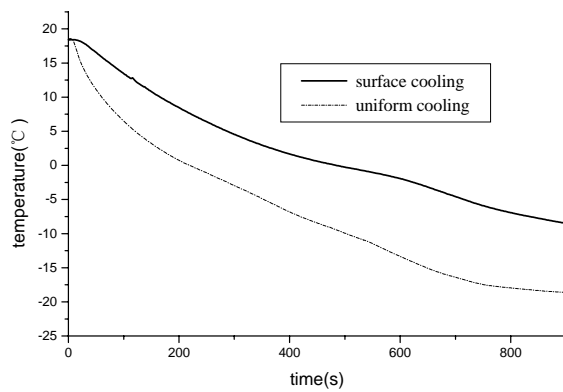
Figure 7 Temperature history for the center point of block A and block B

is obtained in the center similar to other regions of the tissue by using this uniform cooling method, and no evident phase change region exists in the curve. That implies that the tissue by using uniform cooling method may experience a shorter phase change stage, which will reduce the freezing injury of the tissue. Note that in both blocks, the phase change stage was relatively short compared to the computational results. This may be caused by the relatively small size of the block, which dramatically affects the consequence of the cooling process. All the results above imply that uniform cooling method can achieve a rather uniform temperature distribution inside the tissue, and freezing injury caused by large thermal stress due to large temperature gradient will thus be significantly decreased.

DISCUSSIONS AND CONCLUSIONS

This study proposed a uniform cooling method by perfusing cold fluid into micro-pipes specifically constructed over the tissues during culture. Its validity and advantages over the commonly used surface cooling method and limitations are discussed. It should be pointed out that the model of the four parallel pipes in this paper is just for illustration. Actually, various structures (especially the special net works structure) of the pipes can be adopted to meet the heat transfer request. But on the other hand, introducing of such pipes into tissues will increase the complexity of culturing tissues. So the selection of the pipes must be cautious and the biological effect caused by the pipes should also be further discussed. The possibility of using such pipes as channels to load CPA is another aspect worthy of consideration. Besides, combining the nutrition transport in culture as well as flow cooling in later preservation by using the similar pipes is also worth of trying in later study. Clearly, with the development of the tissue engineering technology, three-dimensional tissue culture in large scale would come true in the near future. This uniform cooling method may open a new strategy for successful cryopreservation and thus promise the tissue engineering in culturing large-scale biological objects.

ACKNOWLEDGEMENT

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