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Mechanical and Thermal Damage Accumulation and Recovery in Cell and Tissue Products

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1 Introduction

There are many new and exciting tissue therapies being developed where tissue is grown *in-vitro* and subsequently inserted surgically. For most of these therapies there is considerable distance between the place where the tissue is grown and the hospital where surgery is performed. Assessing how to transport the tissue to ensure the tissue is delivered in a well maintained condition is an important part of making these therapies economically viable. This project considered possible methods of transporting tissue and the possible degradation that might occur in transit. The ability of the method used in transport to mitigate against damage caused by variability in transport conditions, such as temperature or time of transit or against physical damage during to handling errors are important benefits that need assessing.

Artificially grown tissue is transported in relatively small volumes, with typical dimensions of $5 \times 4 \times 0.5$ cm, in bags containing the tissue and some suitable fluid growth medium. Transport tends to be in two main categories namely chilled tissue and frozen tissue. The Study group discussed both of these but concentrated in chilled tissue. The frozen tissue has an extensive literature motivated by such financially important industries such as artificial insemination of cattle and tends to concentrate on damage to cells caused by poor control of the speed of freezing and thawing.

Chilled products are typically transported in the temperature range 2-8^o C. In this range there are no ice crystals formed and so damage to cells can be caused by a number of other different mechanisms. Accepted wisdom indicates that damage to the tissue have the following form as measured by the stated method

- Cell membrane tearing - as identified by dye ingress into cells
- Lack of cell viability - as identified by RNA expression
- Lack of cell viability - as identified by specific markers
- Other problems - as identified through Quality Control using specific markers

Because of the methods used to quantify damage it is not currently possible to identify where it occurs in the transport process, such as during chilling, during holding at a chilled temperature, or during re-heating the tissue. Hence in the modelling done at the study group the work concentrated on the long period when the tissue is transported and relatively short timescale events of cooling and re-heating were not considered. When tissue is transported it is done either in impermeable plastic bag, or permeable plastic bag similar to those shown in Figure 1.

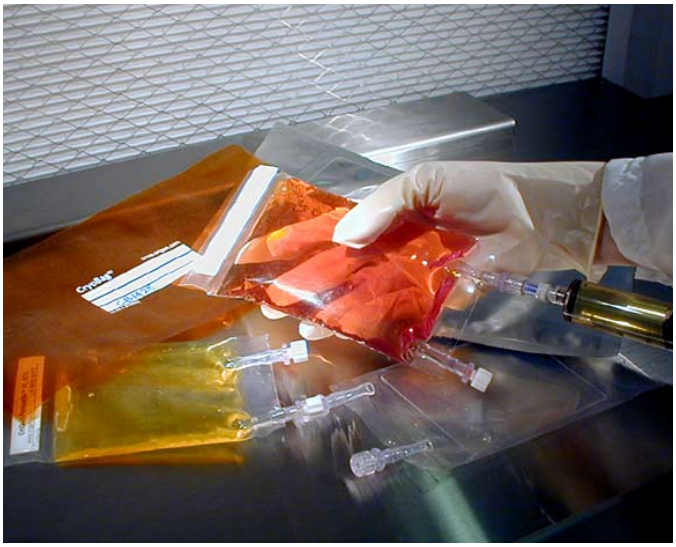

Figure 1: Gas permeable bags typical of those used for tissue transport

1.1 Factors of importance in packaging design

There are a number of important factors to consider in deciding how best to transport a particular tissue. The main factors were

1. Amount of tissue in bag and geometry (tissue width/length/thickness)
2. Density of cells within tissue (the tissue used is typically far from densely packed with cells)
3. Amount of additional fluid medium in bag
4. Amount of additional gas in bag
5. Gas permeability of bag
6. Cell activity
   - O_2 consumption
   - Glucose consumption
   - pH due to lactic acid and cell waste products
   - Growth factors
7. Rate of decay of various product within the serum

In order to help in assessing these factors it was decided to concentrate on a few and hence gain insight into the general type of behaviour before considering the full set of factors. Hence we considered the first five factors (which are relatively easy to manipulate in practice) and to see how these affected the oxygen availability for the cells by accounting for oxygen consumption due to cell activity.

Variable	value	units	Notes
D	$1.7\times\ 10^{-9}$	m^2/s	Diffusion coefficient of O_2
N	6×10^{11}	cells/m^3	Tissue cell density
Q_{O_2}	10^{-16}	mol/cell/s	at 37^oC ($\approx 3 \times 10^{-15}$ g/cell/s) [1]
Q_{O_2}	3×10^{-18}	mol/cell/s	at 5^oC ($\approx 10^{-16}$ g/cell/s) [1]
V_T	10	cm^3	Typical tissue volume (from 5×4×0.5 cm slab)
V_F	5	cm^3	Typical fluid volume
A	20	cm^2	Approximate bag surface area
C_0	7	g/m^3	Initial mass density of O_2 in water (≈ 0.2mol/m^3)
P_{O_2}	0.2	atm	Partial pressure of oxygen in air
K	2.4×10^{-2}	m^3atm/g	Solubility of O_2 in water
κ	2.5×10^{-5}	g/m^2/s/atm	Bag permeability (from 100cc/100in^2/day/atm ! [2])

Table 1: Table showing the modelling parameters and approximate values based on the dimensions of typical transported tissue and data from literature.

2 Basic model of oxygen

We consider the distribution of oxygen within a sample of tissue placed in a plastic bag. Our approach is to use a very simple model and to try to consider "worse-case" conditions in order to give guidance on expected behaviour. We start by considering the distribution of oxygen within the tissue itself.

2.1 Diffusion of oxygen in tissue

The main transport mechanism for oxygen within the tissue is diffusion and as the oxygen is transported it will be consumed by cells as part of their regular metabolic processes. The consumption rate of cells is highly dependent on the cell type and the local environment but we consider the worse case where this rate is a constant independent of other conditions (typically oxygen consumption will decrease with local oxygen levels but we shall not consider this and therefore assume zeroth order reaction kinetics). If we consider a one-dimensional slice of tissue with x being the variable indicating the distance from the tissue surface and C is the concentration of oxygen then the governing equation is

$$D\frac{d^2C}{dx^2} \ = \ Q_{O_2}N$$

with $C = C_0$ at surface of tissue $x = 0$. In this equation we have introduce the constant parameters D, the diffusivity of oxygen in the tissue, N, the cell density in the tissue and Q_{O_2}, the consumption rate per cell. For a number of different cell types these quantities have been measured experimentally at different temperatures [1]. There is a reasonable range of variation in these constants but typical values are shown in Table 1. An Arrhenius relation can be used to give good agreement for temperatures within the range and details of these can be found in [1].

If we take the tissue to be sufficiently thick then the distribution of oxygen is given by a parabolically decaying concentration from the surface until there is no oxygen at which point the consumption

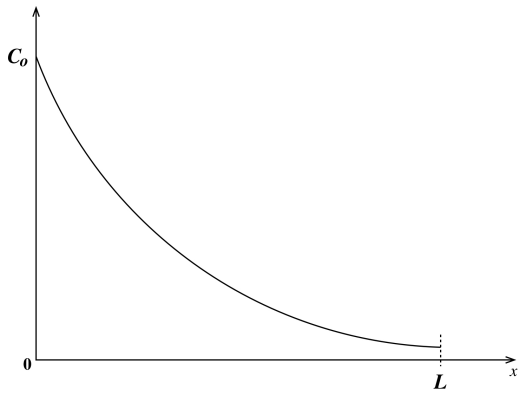

Figure 2: A typical oxygen distribution through the depth of a tissue

ceases. This distribution is sketched in Figure 2.

Solving this problem we find that the distance L from the surface, where the oxygen level would fall to zero is given by

$$L \;=\; \sqrt{\frac{2C_0D}{Q_{O_2}N}}\,.$$

Hence using the data from above we find that:

at 37^oC	$L \approx 0.3$ cm
at 5^oC	$L \approx 2.0$ cm

In practice typical tissue is 0.5cm thick so when the bag is at a chilled temperature oxygen diffuses easily through tissue and there is therefore no significant gradient across the tissue. In addition we note that any additional fluid in bag will be well mixed because of any motion of the bag in transit. We therefore conclude that for any reasonable sample of tissue in a bag we can assume that the oxygen concentration is almost uniform throughout the bag. We will now use this assumption to determine how the oxygen concentration changes with time.

2.2 Model of oxygen in Bag

Having determined that in most practical situations the concentration of oxygen is nearly spatially uniform throughout the bag and derived expressions for when this approximation is realistic we now consider how this spatially uniform concentration varies with time. For simplicity we assume that there is no additional gas in the bag, so that all the oxygen is in the medium and tissue, but this can be accounted for relatively easily if necessary. We take the bag to have concentration of oxygen $C(t)$ and for the volume of tissue to be V_T and the volume of extra fluid to be V_F. A very rough stretch of the bag is given in Figure 3

Oxygen in the bag is consumed by the cells but can be replenished by transport across the permeable bag surface. The oxygen outside the bag is assumed to be at standard conditions. The equation for conservation of oxygen in the bag is therefore

$$\frac{d}{dt}\Big(\,(V_T + V_f)\,C\Big) \;=\; -\,Q_{O_2}NV_T + \kappa A(P_{O_2} - KC)$$

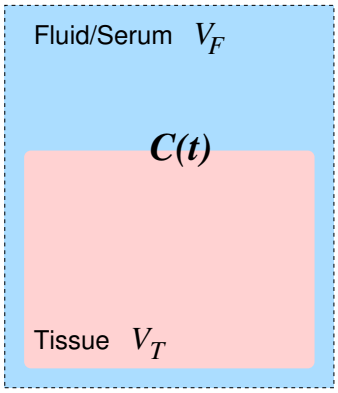

Figure 3: A very rough schematic of tissue (volume V_T) in fluid/serum filled bag (fluid volume = V_F).

with $C(0) = C_0$. A brief description of the parameters as well as approximate values, based on current practice, are listed in Table 1.

This simple first order linear ordinary differential equation for $C(t)$ can be easily solved and the solution then exploited to understand the behaviour of the oxygen in any particular situation. The solution is

$$C = \frac{\kappa A P_{O_2} - Q_{O_2} N V_T}{\kappa A K} \left(1 - \exp\left(\frac{-\kappa A K}{V_T + V_F}\, t\right)\right) + C_0 \exp\left(\frac{-\kappa A K}{V_T + V_F}\, t\right)$$

The main practical question we would like to consider is: How do we choose the parameters under our control so that $C > 0$ for the entire time the tissue is in transit (≈ 2 days)?

It is worth considering a special case that is of practical interest namely transport of tissue in an impermeable bag (this corresponds to the case where $\kappa = 0$). The time, t_d, for all the oxygen to be used up and for the concentration to drop to zero in the bag is then

$$t_d = \frac{C_0(V_T + V_F)}{Q_{O_2} N V_T}$$

and using typical data we conclude that this time is:

at 37^{o}C	≈ 2 hours
at 5^{o}C	≈ 2 days

showing that the use of inexpensive impermeable bags is viable for many situations but that there is little margin for error if there are delays or if the temperature is not regulated well. It also shows that the added fluid medium only extends the time by about a factor of two over tissue transported with no extra fluid.

If a permeable bag is used then there will always be some oxygen in the bag, however it is instructive to understand how tolerant the system will be to external variations. Two aspects are worth noting, first there is timescale for the gas in the bag to equilibriate with the outside oxygen so that the bag is then at a constant oxygen level and the second is to determine how far below atmospheric oxygen levels this steady state condition is. In practice reliable transport could be

attained either by having a very long equilibrium time, so that oxygen levels stayed high for most of the expected transit time or to have the steady state level high so that the bag was always at a high level independent of the transit time. Using the data for a typical permeable bag we find that at 5^oC:

Timescale for equilibration:	≈ 3 hours
Fraction below atmospheric solubility in steady state:	≈ 0.06

Hence in practice the current expensive permeable bags are very good because their steady state level of oxygen is 94% of that in normal atmosphere.

3 Other aspects

There was some discussion of some of the other aspects of the bag design that might be important. The modelling framework outlined above is directly appreciable to considering the distribution of glucose and other nutrient within the bag. No precise calculations were performed but we anticipate that, although such nutrient are slightly less diffusable that oxygen we would still expect that at chilled temperatures the distribution will again be almost uniform. If data on consumption of nutrient at low temperatures is not available we would expect it to be closely related to the oxygen consumption rate. The balance equation for the nutrient would then be similar to that for oxygen but with no transfer from the outside world. In such cases, because the steady state corresponds to no nutrient, we would expect that the additional fluid medium to be crucial in extending the time before the nutrient levels reach unacceptably low levels (this would be equivalent to using this volume to increase the equilibration time).

4 Summary

The variation in oxygen in a tissue stored in a plastic bag has been considered. We have found that at typical temperatures used for such chilled products the distance that oxygen diffuses into tissue before being completely consumed is around 5 cm. Hence for most situations the concentration of oxygen in the tissue will be spatially uniform and that the surrounding fluid in the bag will be at the same concentration. Analytical expressions have been derived that allow the validity of these approxiamtions in any particular situation to be readily assessed.

By considering different types of bag material we have found that impermeable bags will allow thin tissue, about 5mm thick, to retain oxygen for about 2 days (extra fluid is not really necessary) Commercially available permeable bags make oxygen levels in the bag go to a steady state and for typical chilled conditions will ensure oxygen levels stay high for tissue as thick as 5cm

Glucose and other nutrients within the bag can be modelled in the same framework but no details have yet been derived. However, because there is no transfer of nutrients from outside and because we anticipate slightly shorter diffusion lengths than for oxygen there will be a need to provide extra fluid medium to have sufficient nutrients for transport.

The modeling done so far gives some insight into packaging for reliable transport. There are a number of other issues that might also need considering such as the temperature history and

particularly the rate of cooling and rate of heating, but these will require further experimental evidence in order to assess their importance.

References

[1] Jorjani, P., Ozturk, S.S., Effects of cell density and temperature on oxygen consumption rate for different mammalian cell lines, Biotech and Bioeng, **64 (3)**, 349–356, (1999).

[2] Avgoustiniatos, E.S., et al., Commercially available gas-permeable cell culture bags may not prevent anoxia in cultures or shipped islets, Transplantation Proc., **40**, 395–400, (2008).