

TRANSPORT CHARACTERISTICS OF GLYCEROL AND PROPYLENE GLYCOL IN AN ENGINEERED DERMAL REPLACEMENT

A. Hubel*¹, N. Bidault **, B. Hammer[#]

Departments of Laboratory Medicine and Pathology* and Radiology[#], University
of Minnesota, Minneapolis, MN 55455

**Proctor and Gamble Corporation, Rome, Italy

INTRODUCTION

The ability to cryopreserve engineered tissues is important for the clinical application of therapies based on living cells. Cryopreservation facilitates the manufacture, transport and safety of cell-based therapies. The cryopreservation of cells and tissues had typically required the use of specialized solutions containing cryoprotective agents (CPAs). The addition of a CPA to the freezing solution may result in damage if it is not done properly. Tissues and intact organs can exhibit reduced cellular viability when exposed to sufficiently large step changes in external osmolarity resulting from introduction or removal of a cryopreservation solution (Pegg, 1972). Not only are large step changes in osmolarity potentially damaging, but also long-term exposure to even low concentrations of CPAs at room temperature can be lethal (Fahy et al., 1990). Exposure of cells to CPAs (in particular dimethyl sulfoxide, Me₂SO) has been associated with a loss in viability with time of exposure. Subsequent studies have quantified specific cellular changes resulting from exposure to CPA, such as cytoskeletal reorganization, cross-linking of nuclear proteins, and alterations in membrane permeability (cf. ref (Fahy et al., 1990) for review) which may account for the loss in viability.

Nuclear Magnetic Resonance (NMR) is a common nondestructive technique used to quantitate the concentration of molecules inside 3D structures. Several MR techniques have been developed to quantitate the concentration of CPAs in tissues during permeation. Spectroscopic techniques have been used (Fuller et al., 1989) to determine average concentrations with time. MRI has also been used to image CPA permeation in zebra fish embryos (Hagedorn et al., 1998) or mammalian organs (Isbell et al., 1997).

We have developed a rapid imaging technique that can be applied to the study of the permeation of CPA in artificial tissues (Bidault et al., 2000; Bidault et al., 2001). A combined Fast Low Angle Shot (FLASH)-Keyhole technique was used to increase the speed and sensitivity of images obtained of CPA permeation in tissue. This technique was used to quantify the water and Me₂SO content of an engineered dermal replacement as a function of temperature, time and bulk CPA concentration. In this study, we extend this analysis to

include glycerol and propylene glycol, two CPAs also commonly used for tissue preservation.

MATERIALS AND METHODS

Culture of the dermal replacement: Collagen scaffolding was used to support the growth of the dermal fibroblasts and the formation of a dermal equivalent. Based on a protocol described in more detail in ref. (Bidault et al., 2001), the scaffold consisted of 0.5% w/v collagen from bovine corium (Kensley-Nash, Exton, PA) mixed with 1:19 w/w % hyaluronic acid (Lifecore Biomedical, Inc., Chaska, MN) and dispersed in H₂O+HCl solution at pH=3.0. The dispersion was freeze dried, crosslinked and sterilized prior to use. In order to reconstitute a dermal replacement, the collagen sponge was seeded with normal human dermal fibroblasts (Clonetics, Inc., San Diego, CA) cultured in Dulbecco's Modified Eagles Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% Fetal Bovine Serum (FBS) (Hyclone, Logan, UT), 100 units/mL penicillin G and 100 mg/mL streptomycin (Gibco) and 10% w/w ascorbic acid (Gibco). The artificial dermis was cultured at 37 °C and a 5% CO₂ atmosphere for 14 days with media changes every other day.

Diffusion coefficient measurement: NMR spectroscopy was used to determine the diffusion coefficient of glycerol and propylene glycol in solution and in the dermal equivalent. Determination of the diffusion coefficient in solution can be used as an indicator of possible interactions such as hydrogen binding. When performed in a tissue, these measurements can provide information on additional interactions such as structural binding. The method of determining diffusion coefficient can be found in more detail in ref. (Le Bihan, 1995). Briefly, a spin echo pulse sequence is used in combination with a pair of gradients on both sides of the 180° pulse. The echo amplitude decay was measured and the value of the diffusion coefficient was determined from that signal.

¹ Corresponding author. E-mail: hubel001@umn.edu

RESULTS AND DISCUSSION

Quantifying the transport of CPAs in engineered tissues requires the determination of the diffusion characteristics for these molecules. The first phase of this investigation involved determination of the diffusion characteristics of glycerol and propylene glycol in water.

Spectrum: Glycerol is a three-alcohol molecule whose NMR spectrum shows two peaks, a CH₂- and CH peak. The two peaks are very close to each other and are frequently difficult to distinguish (fig 1a). Propylene glycol possesses one CH₃-, one CH₂- and one CH-group per molecule. As such three different peaks are visible in the NMR spectrum (fig 1b).

Diffusion coefficient: The diffusion coefficient of glycerol and propylene glycol in water was determined as a function of CPA concentration (fig. 2). The diffusion coefficient for the CPA in water decreases with increasing CPA concentration. The self-diffusion coefficient for water in these solutions was higher than that of the CPA ($0.5\text{-}2 \times 10^{-5} \text{ cm}^2/\text{s}$) and the value of the diffusion coefficient for water decreased with increasing CPA concentration.

CPA permeation: the permeation into the dermal replacement of a glycerol+balanced salt solution was imaged for concentrations of glycerol ranging between 20-40 % v/v. Lower concentrations could not be imaged due to problems with signal/noise. The imaging time for that range of glycerol concentrations ranged between 96 and 175 secs. A similar range of imaging times and concentrations that could be imaged was observed for propylene glycol.

In order to determine the influence of the matrix on the diffusion of CPA in the engineered dermis, we also determined the diffusion coefficient of glycerol or propylene glycol in water in a freeze-dried dermal replacement. Measurements of the gradient factor indicated that the CPA present in the matrix freely diffuses and the proportion of CPA molecules with restricted diffusion was limited. In contrast, the gradient factor determined for water indicates that a significant fraction of water molecules are restricted in the tissue. This result is consistent with a study of water diffusion in type II collagen (Knauss et al., 1996). The diffusion coefficient for a solution containing 40 % v/v glycerol in water in a freeze-dried tissue was approximately $2 \times 10^{-6} \text{ cm}^2/\text{s}$. The diffusion coefficient in turn can be used to facilitate the development of models of the diffusion of water and CPA in engineered tissues.

REFERENCES

- Bidault, N.P., Hammer, B.E. and Hubel, A., 2000. Rapid MR imaging of cryoprotectant permeation in an engineered dermal replacement. *Cryobiology*, 40(1): 13-26.
- Bidault, N.P., Hammer, B.E. and Hubel, A., 2001. Water content in an engineered dermal replacement during permeation of Me₂SO solutions using rapid MR imaging. *Biotechnol Prog*, 17(3): 530-6.
- Fahy, G.M., Lilley, T.H., Linsdell, H., Douglas, M.S. and Meryman, H.T., 1990. Cryoprotectant toxicity and cryoprotectant toxicity reduction: in search of molecular mechanisms. *Cryobiology*, 27(3): 247-68.
- Fuller, B.J., Busza, A.L. and Proctor, E., 1989. Studies on cryoprotectant equilibration in the intact rat liver using nuclear magnetic resonance spectroscopy: a noninvasive method to assess distribution of dimethyl sulfoxide in tissues. *Cryobiology*, 26(2): 112-8.
- Hagedorn, M., Kleinhans, F.W., Artemov, D. and Pilatus, U., 1998. Characterization of a major permeability barrier in the zebrafish embryo. *Biol Reprod*, 59(5): 1240-50.
- Isbell, S.A., Fyfe, C.A., Ammons, R.L. and Pearson, B., 1997. Measurement of cryoprotective solvent penetration into intact

organ tissues using high-field NMR microimaging. *Cryobiology*, 35(2): 165-72.

Knauss, R., Fleischer, G., Grunder, W., Karger, J. and Werner, A., 1996. Pulsed field gradient NMR and nuclear magnetic relaxation studies of water mobility in hydrated collagen II. *Magn Reson Med*, 36(2): 241-8.

Le Bihan, D., 1995. *Diffusion and Perfusion Magnetic Resonance Imaging. Applications to Functional MRI*. Raven Press, New York.

Pegg, D.E., 1972. Perfusion of rabbit kidneys with cryoprotective agents. *Cryobiology*, 9(5): 411-9.

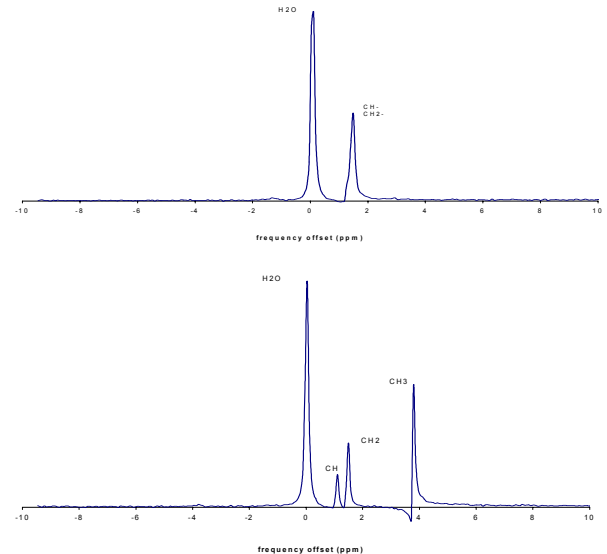


Figure 1. NMR spectrum for (a) glycerol and (b) propylene glycol.

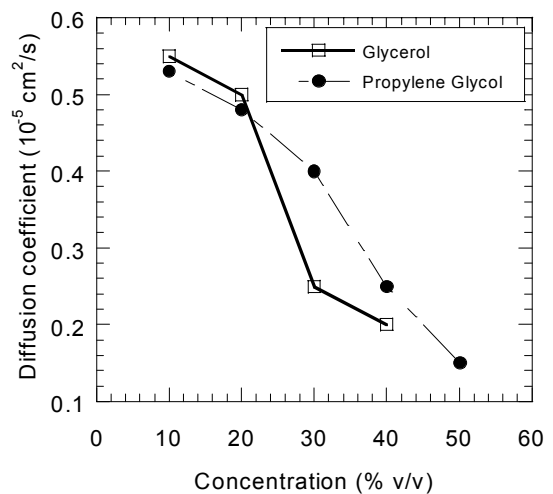


Figure 2. Diffusion coefficient for glycerol and propylene glycol in water for different concentrations of CPA at 19 °C.