THE STORAGE OF HUMAN TISSUES FOR SURGICAL USE

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The replacement of a diseased or an injured anatomical part, such as bone, skin, fascia, dura, cartilage or artery, by stored human tissue constitutes a major advance in the field of traumatic and reconstructive surgery. The concept of tissue storage is not new. Dr. Alexis Carrel, in 1912, stated: 'It would be very convenient for the surgeon to keep in store pieces of skin, periosteum, bone, cartilage, blood vessels, peritoneum, omentum and fat ready to be used.' Today, the developments of this concept have reached such proportions that their principles approach those of a new discipline in surgery.

Basically, the surgeon's requirements for a useful stored human tissue are that the tissue, when grafted, will perform predictably and with reliable reproducibility. This successful performance depends upon the accomplishment of certain biodynamic functions of the graft, which are:

1. Biological acceptance of the graft by the host.
2. Dynamic alteration in both the graft and the host.
3. Mechanical competence in accepting physiological demands.
4. Provision of a template for ingrowing host tissue with eventual complete replacement by host tissue in the anatomical form of the graft.

Freezing

The freezing of human tissue should achieve, ideally, relative molecular immobility without any alteration in the biological activity of the tissue. Upon thawing, the tissue should resume completely its temporarily arrested metabolic activity and related functions. Unfortunately, all of the freezing methods currently used for the storage of tissue fall short of this ideal. Our failure centres around two main problems, namely:

1. We cannot apply the freezing velocities which have been found by the biologist to be necessary for the survival of isolated cells or small cell masses to the incomparably larger masses of human tissue required in the various operative procedures.
2. During slow freezing, the liquid phase of tissue electrolytes probably changes to hypertonic salt solutions, or 'tissue brine,' which is lethal to the cells and potentially destructive to the matrix.

Some of the more important problems currently under investigation are:

1. The significance of ice recrystallization in the solid state.
2. The importance of extra- and intra-cellular ice crystals in relation to cell viability.
3. The importance of tissue-electrolyte concentrations and cell damage.
4. The significance of 'protected' freezing, using glycerol, lactose, and other similar substances.
5. The effect of freezing on lipoprotein complexes within the cells.
6. The existence and significance of thermal shock to the cells.
7. The changes in cell enzyme systems with freezing.

Despite these difficulties in the investigative field and the many remaining unanswered questions, the use of the 'deep freeze' to store human tissues for surgical use remains a relatively simple and practical method for limited short-term storage.

Freeze-drying

The principle of freeze-drying employs the concept of removing tissue ice by sublimation in a vacuum. This is calculated to retain the biodynamic capacities of the tissue and still permit shelf storage at room temperatures. From the clinical standpoint it is not necessary nor is it particularly desirable to maintain cell viability in the adult homograft, with the possible exception of hyaline cartilage. Bone was the first tissue to be freeze-dried and used clinically. The processing method was developed by Flosdorf and
The advantages of freeze-drying are:
(i) The method employs what is theoretically the least destructive principle that can be applied to tissue and still achieve long-term storage.
(ii) The final dried product may be stored at room temperature.
(iii) There is no loss in form or shape of the final product.
(iv) Tissue shipment from one area to another in time of necessity is more easily accomplished.

The disadvantages of freeze-drying are:
(i) High initial expense of the equipment.
(ii) The delay involved in processing the tissue before storage.

Technique of Freeze-drying

The tissues to be freeze-dried are usually obtained under aseptic conditions. The tissues are frozen and stored at −60°C awaiting their turn in the production cycle. The freeze-drying unit currently in use in our bank is a large standard-chamber design (Fig. 1). The two hollow condenser plates are cooled, using Freon as a refrigerant, maintaining a constant condensing-surface temperature of −45°C. The upper hollow plate can be either cooled or warmed, using trichlor-ethylene as the circulating fluid. This permits adjustments in the temperature of this drying plate for more efficient drying.

To start the freeze-drying cycle, the interior of the chamber is sterilized with a chemical sterilizing agent. A mixture of dry ice and alcohol is placed in an external cooler so that the temperature of the drying plate approximates the temperature of the condenser plate at −45°C. With aseptic precautions, the tissues to be deposited are removed from the deep-freeze unit, unwrapped, and placed on the drying shelf of the unit. The unit is closed and the vacuum unit started. After approximately 30 minutes, the vacuum is constant at 5 to 10 microns. The temperature of the upper, or drying, shelf is allowed to rise gradually to 0°C within 24 hours and to +30°C on the ninth day in order to utilize latent heat in obtaining more efficient drying. The temperature of the lower shelves remains at −45°C. On the fourteenth day the cycle for bone is considered complete. The period of time is undoubtedly in excess
Fig. 2.—Capping unit.

Fig. 3.—Shelf storage with units.
of that required for drying, but we are encouraged to maintain the time relationship in view of the variability of the load, the erratic residual moisture of the bone, plus the clinical success that we have had with this system.

The deposits are removed from the unit, a secondary vacuum of approximately 3,000 microns drawn, and the individual deposit capped (Fig. 2). A secondary vacuum-tight seal is made by dipping the top of the bottle and the cap in hot wax. The freeze-dried deposit is appropriately labelled and placed on the storage shelf at room temperature.

The acceptable endpoint of the freeze-drying cycle has been somewhat arbitrarily determined by assessing the amount of residual moisture remaining in the graft. This determination is based on the observation of Flosdorf which indicated that the residual moisture of freeze-dried sera was an index of its keeping qualities. Residual moisture levels of from 2 to 5 per cent. have been achieved and accepted in our laboratory, utilizing a recently developed technique. Increasing the duration of the freeze-drying cycle has not resulted in a significantly lower final moisture content. Bone stored for five years with a level of 2 to 4 per cent. residual moisture (Fig. 3) has been used clinically with no gross evidence of unusual host responses.

**Nutrient Media**

Nutrient media storage takes advantage of the lowered cell metabolism and decreased oxygen demands produced by lowered temperatures. Storage is usually in the region of $+4^\circ$ C, based on the findings of Hanks that at $6$ to $8^\circ$ C more oxygen is required by the cells to support metabolism than at $0^\circ$ C. The media provides for cell nutrition at the depressed level of metabolic activity. Furthermore, the buffering capacity of the media takes care of the accumulating cell catabolites. An indicator of excessive catabolite accumulation, usually phenol red, is used to determine the need for a change in the nutrient media solution. To avoid the deleterious effect on the tissue cells of a complete change of media, as noted by Allgower and Blocker, one-half of the previously used nutrient media is left in the deposit unit. Pierce et al. found that dog arteries in nutrient media storage (2 to 6 cm. sections per 20 ml. media) maintained viability, as determined in tissue culture, for seven weeks. Human split-thickness skin stored in nutrient media in our laboratory (Fig. 4) has shown survival for six weeks. There was no survival after eight weeks under any of our laboratory conditions, using an average of 2.4 ml. of nutrient media per square centimetre of skin.

While the theoretical oxygen-temperature relationship and the required solution-tissue volume ratio have been determined, their ideal remains to be demonstrated in so far as general surgical utilization is concerned. Nutrient media is used to maintain tissue viability and to provide a
simple method of short-term storage. Viability, as ordinarily determined in tissue culture, probably is a biological indicator of the preservation of the important enzyme systems.

The nutrient media currently in use in the tissue bank consists of 90 per cent. balance salt solution (Earle's) and 10 per cent. human serum with added pH indicator in the form of phenol red.

One of the advantages of nutrient media storage is that it lends itself to use by hospitals wishing to trans-ship viable tissues for final processing. We have satisfactorily processed over 300 arteries shipped to us by air from other hospitals. These arteries were sent in nutrient media in refrigerated packages, freeze-dried in the tissue bank, and then returned to the hospital from which they were sent.

Protected Freezing

In 1949, Polge, Smith and Parkes discovered that glycerol and related compounds have the unusual property of protecting cells against the usual effects of freezing. Mollison and Sloviter, in 1951, transfused human blood stored for several hours at -79°C following protection of the cells with 15 per cent. glycerol. They found that there was about 50 per cent. haemolysis by this method but that the recovered cells survived normally. By further experimentation, Chaplin and Mollison in 1953 were able to decrease the haemolysis to about 2 per cent., using glycerol in a concentration of 30 per cent. There is some evidence to suggest that despite glycerol protection there is some cell deterioration which results from storage at very low temperatures, although Smith and Parkes, in studying ovarian tissue stored at -190°C C, found no greater damage at one year than at one month. While the mechanism of glycerol protection is not completely understood, its application is probably concerned with the following:

1. The freezing-point depression and osmotic pressure changes involving solutions of glycerol and cell electrolytes.
2. The hygroscopic property of glycerol and the prevention of complete dehydration of the cells.
3. The possible partial protection that glycerol has in the prevention of lipoprotein denaturation.

Protected freezing for long-term storage has not found widespread use with the possible exception of the blood preservation programme. Considering the current level of experimentation in the field of protected freezing, we may anticipate that this aspect of storage will offer greater possibilities in the future.

Chemical Antiseptics

Merthiolate is the only one of the group of chemical antiseptics that is in continued use at the present time, largely on an empirical basis. It is used in the aqueous form, primarily in the storage of cartilage. The disadvantage of using merthiolate is that it results in the loss of cellular viability. This disadvantage is not offset by any advantages over other storage systems with the result that the method of merthiolate storage has not received widespread use.

Results

The tissue bank at the United States Naval Medical Centre in Bethesda, Maryland, is a clinical research and development project which is concerned, primarily, with the stored human homograft. Some of the clinical results which have been obtained using these stored grafts are as follows:

Bone.—Over 1,800 patients have been grafted with freeze-dried bone homografts. Some of the homografts had been stored over five years prior to their use without evidence of undue reaction on the part of the host. The results of these grafts in 1,868 patients are shown in Table I. In 550 cases which have been adequately followed to permit determination as to their success or failure, there were 394 cases classified as successful. Of the remaining 156 cases, 96 were classified as technical failures and 60 were classified as graft failures. The best results were obtained in the treatment of fresh fractures and the poorest results obtained when the grafts were used in cases where infection existed at the time the grafting procedure was carried out.

Fascia.—Freeze-dried fascial homografts have been used surgically in 149 patients. The fascia has been used in such procedures as hernia repairs, fascial slings, radical breast repairs, synostosis, orbicular ligament repairs and others. The initial clinical accounting, as reported by Snyderman, appears to be favourable.

Skin.—The various storage methods, as applied to split-thickness skin grafts, have been sufficiently satisfactory to permit clinical use in the treatment of 204 patients. A detailed and critical analysis of information on the treatment of 43 critically burned patients, made available to the tissue bank by its collaborators, is to be published in the near future. Certain generalizations can be made at the present time; they are as follows:

1. Freeze-dried skin has the most satisfactory initial 'take' as compared to skin stored in nutrient media and skin stored by glycerol-protected freezing.
2. Skin stored in nutrient media has the longest 'stay' period.
(3) The more vigorous the initial treatment in attempting to cover the burn area with skin, regardless of the method of storage, the less the morbidity and mortality.

Arteries. — Eighty-nine patients have been grafted with freeze-dried arteries which were procured and processed by the Tissue Bank. These vessels were implanted by the surgical staff at the Naval Hospital in the National Naval Medical Centre. The initial results were reported by Brown et al.16

Dura. — Freeze-dried dura has been used in 70 cases. The use and fate of the graft is discussed by Campbell, Bassett and Robertson.16

Discussion

In clinical experience, the grafts which are most commonly stored for surgical use are the homografts. The biodynamic functions of the homografts are most competently exercised when they are used as orthotopic grafts; that is, tissue grafted into an anatomical site normally occupied by the same type of tissue. It is to be noted that the clinical success of the homograft is not always dependent upon the accomplishment of the graft’s biodynamic functions. For example: it is generally agreed that an arterial homograft does not induce the host to form new elastic fibrils, but rather functionally-oriented collagen is the final replacement and it is haemodynamically satisfactory. The ultimate functional result has not been determined with respect to duration of time. Even with the unsuccessful graft, as with the use of homograft skin, we have been able to turn a biological failure into a measure of clinical success. It should be stressed that the autograft remains clinically superior to the stored homograft. The stored homograft plays its part in surgery only when the autograft is not reasonably available.

Summary

(1) The basic requirements of a useful stored graft are defined and explained in the biodynamic graft capacities.

(2) The broad principles of freezing, freeze-drying, glycerol-protected freezing, nutrient media and chemical antiseptics, as applied to tissue-graft storage, are presented.

(3) The clinical results as applied to stored homografts of bone, skin, fascia, dura and arteries are presented as indications of surgical trends.

BIBLIOGRAPHY

3. KREUZ et al. (1951), J. Bone It Surg., 33A, 863.
4. HYATT, G. W., and BUTLER, M. C. (1957), American Academy of Orthopaedic Surgeons' Instructional Course Lectures.