Replantation of Cryopreserved Fingers: An "Organ Banking" Breakthrough

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Background: Although cryopreservation has been widely used in clinical practice, it remains limited to small or thin bloodless tissues with a simple structure and function. In August of 2002, the authors performed the first successful transplantation of a cryopreserved vascularized rat hind limb. Subsequently, the authors extended this cryopreservation technique to the preservation of human fingers.

Methods: In December of 2002 and December of 2003, the authors performed two in situ implantations of cryopreserved amputated fingers for two patients by means of the "two-step" and programmed cryopreservation methods. In case 1, computed tomographic angiography was performed on the affected hand 6 months after surgery. In case 2, pieces of skin were obtained from the thawed amputated finger for pathologic examination before replantation.

Results: One finger was cryopreserved for 81 days and the other for 5 days. Both fingers were replanted successfully. Computed tomographic angiography revealed a patent radial proper digital artery in case 1. The pathologic results of case 2 showed satisfactory skin cell morphology. After 15- and 14-year follow-up assessments of the two patients, the replanted fingers achieved satisfactory appearance and function.

Conclusions: The authors confirmed the effectiveness of deep cryopreservation for the long-term preservation of human fingers. The current application scope of these cryopreservation techniques includes small limbs with minimal amounts of muscle tissue. (*Plast. Reconstr. Surg.* 144: 679, 2019.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, V.

Although cryopreservation has been widely used in clinical practice, it remains limited to bloodless tissues with a simple structure and function that are small or thin. Except for the cryopreservation successes of the rat ovary (2002) and hind limb (August of 2002), successful cryopreservations and replantations of vascularized animal tissues or whole organs have not been reported. In December of 2002 and December of 2003, we successfully replanted two amputated fingers after cryopreservation, demonstrating the

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Received for publication May 21, 2018; accepted February 4, 2019.

DOI: 10.1097/PRS.0000000000005979

Disclosure: *The authors have no conflicts of interest to declare.*

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A "Hot Topic Video" by Editor-in-Chief Rod J. Rohrich, M.D., accompanies this article. Go to PRSJournal.com and click on "Plastic Surgery Hot Topics" in the "Digital Media" tab to watch.

first known successful application of cryopreservation technology in clinical practice. In this article, we hope our experience can provide beneficial knowledge to our colleagues, with the goal of further developing this technology.

In clinical work, it is common for some patients to initially refuse replantation but change their minds at a later time. In addition, some patients with organ failure cannot immediately undergo replantation. Replantation is severely limited by a short preservation time; thus, cryopreservation might possibly solve this problem by extending the preservation period. In 2002, we reported our first success of rewarming and replanting (by means of vascular anastomosis) a rat hind limb preserved using cryopreservation.[1–3] Subsequently, we extended the cryopreservation technique to two human amputated fingers. To confirm the prognosis of the replanted fingers, we conducted a long-term follow-up evaluation. This study was approved by the Institutional Review Board of the Provincial Hospital Affiliated with Shandong University, People's Republic of China. In this article, we hope that our experience can provide beneficial knowledge for our colleagues, with the goal of further developing this technology.

CASE REPORTS

Case 1

On September 24, 2002, a 19-year-old man presented with a completely amputated left index finger. [**See Figure, Supplemental Digital Content 1**, which shows a photographic series of patient 1. (*Above, left*) Dorsal side of the amputated finger. (*Above, second from left*) Palm side of the amputated finger. (*Above, second from right*) Dorsal side of the injured hand. (*Above, right*) Palm side of the injured hand. (*Center, left*) Palm side after reimplantation. (*Center, second from left*) Dorsal side after reimplantation. (*Center, second from right*) Palm side 1 month after surgery. (*Center, right*) Dorsal side 1 month after surgery. (*Below, left*) Radiograph obtained 1 month after surgery. (*Below, second from left*) Radiograph obtained 6 months after surgery. (*Below, second from right*) Function 70 days after surgery (*medial view*). (*Below, right*) Angiograph obtained 6 months after surgery showing blood flow in the transplanted finger (although with a reduced number of blood vessels), ***http://links.lww.com/PRS/D648***. **See Figure, Supplemental Digital Content 2**, which shows a 15-year follow-up visit after surgery. The function of the affected finger was favorable. (*Above, left*) Dorsal side of the left hand. (*Above, second from left*) Dorsal side of the left index finger. (*Above, second from right*) Index-to-thumb opposition of the left hand. (*Above, right*) Fist formation of the left hand. (*Below, left*) Palm side of the left hand. (*Below, second from left*) The patient pinching a ballpoint pen with his left index finger. (*Below, second from right*) The patient holding a mobile phone with his left index finger and thumb. (*Below, right*) Radiographic anteroposterior view of the affected finger, ***http://links.lww.com/PRS/D649***.] He had cut off his left index finger himself after a quarrel with his girlfriend and was transferred to our hospital after simple wound management. He refused replantation of the amputated finger and underwent débridement and closure of the wound. The amputated stump was treated using the routine procedure. We shortened the proximal bone approximately 6 mm and closed the skin directly in fishmouth manner to retain the length of the digital nerves and arteries as much as possible. Then, the patient abandoned the amputated finger and gave our staff permission to dispose of it.

The amputated finger was perfused with a previously described cryopreservation solution[1] (10% fetal bovine serum, 12% dimethyl sulfoxide, and 78% RPMI 1640 medium) through the nondominant (radial) proper digital artery and was stored in a sealed sterile polyethylene bag at in a 4°C refrigerator for 15 hours, a −20°C freezer for 4 hours, and then a −80°C freezer for 24 hours before being placed into liquid nitrogen (−196°C).

Exactly 72 days later, the patient returned to the hospital to request left index finger reconstruction. However, he decided to undergo replantation surgery when he was informed that his amputated finger had been cryopreserved. On December 14, 2002, the patient underwent replantation of his left amputated index finger under a brachial plexus block. The amputated finger was placed in a jar with 300 ml of sterile RPMI 1640 medium in a thermostatic shaking water bath to thaw. During surgery, the musculus flexor digitorum superficialis was intact, and the musculi flexor digitorum profundus was repaired. The primary débridement of the left index stump caused damage to the proper digital artery and dorsal digital vein. Vein grafts were obtained from the superficial veins of the left forearm to reconstruct two proper digital arteries and three dorsal veins. During exploration, we found that the proximal stump of the nerves was slightly enlarged. After removing the enlarged part, the nerves had no obvious defects and were directly sutured. Because this procedure was a second-stage replantation, segmental soft-tissue loss occurred. Thus, the replanted finger was shorter than normal. After surgery, antiinflammatory, anticoagulation, and antispasmodic treatments were applied as usual.

At the 70-day follow-up visit, no worsening of the atrophy of the replanted finger was observed, and the function of the replanted finger was favorable. Six months after surgery, computed tomographic angiography was performed on the affected hand, which showed only that the radial proper digital artery was smooth. At the 15-year follow-up visit, although mild to moderate atrophy of the left index finger was observed, the function of the left index finger was satisfactory, and the patient was able to successfully complete grasping, holding, and pinching actions. Radiography revealed adequate bone healing. The patient achieved 0- to 85-degree active flexion of the proximal interphalangeal joints and 0- to 30-degree active flexion of the distal interphalangeal joints. The static two-point discrimination was 6 mm and the Semmes-Weinstein monofilament test result was 2.83.

Case 2

On December 9, 2003, a 27-year-old man cut off his left index finger by himself under emotional duress and refused to undergo replantation. The perfused amputated finger was placed in a Planer Controlled Rate Freezer (Kryo 560-16; Planer PLC, Middlesex, United Kingdom) for programmed freezing and was ultimately stored in the liquid nitrogen (−196°C).

On December 14, 2003, the patient underwent replantation of his left amputated index finger (Figs. 1 through 3). During surgery, the musculus flexor digitorum superficialis was intact, the musculi flexor digitorum profundus was repaired, and vein grafts were obtained to reconstruct the digital arteries. Before replantation, pieces of skin were obtained from the thawed amputated finger for pathologic examination. The pathologic results showed satisfactory skin cell morphology (Fig. 4).

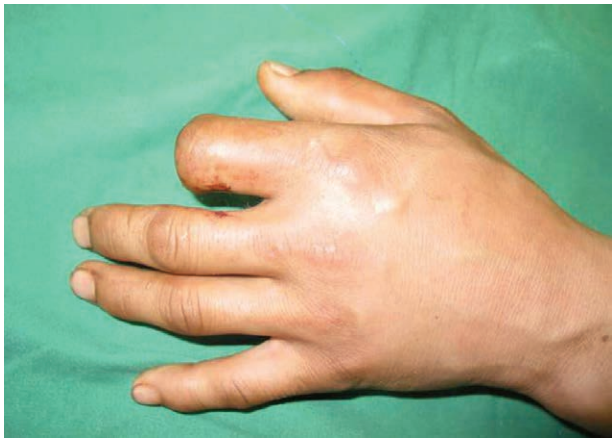

Fig. 1. Dorsal side of the injured hand 5 days after débridement and direct suture (patient 2). The hand wound was healed.

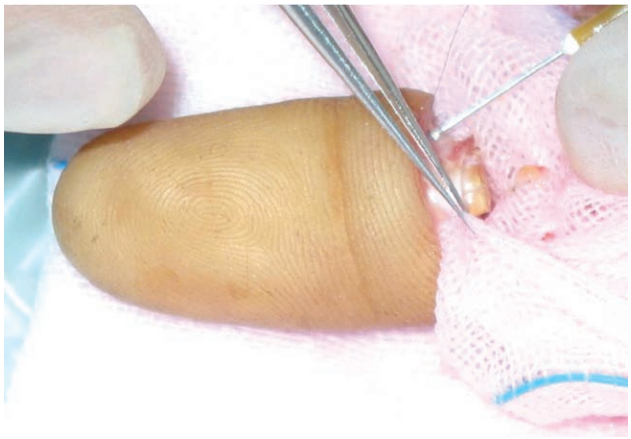

Fig. 2. Irrigation of the amputated finger with heparin saline solution (12,500 U of heparin in 500 ml of normal saline) after being thawed (patient 2).

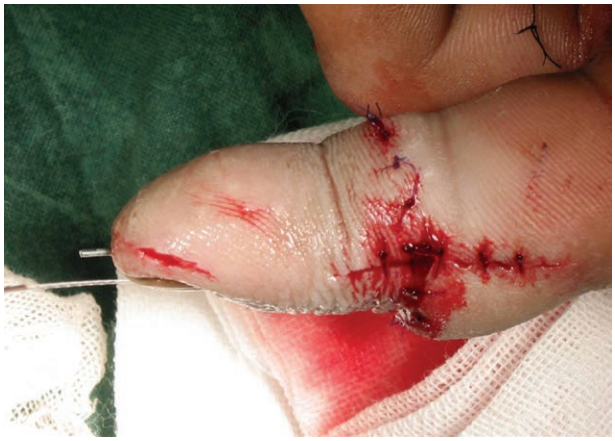

Fig. 3. First day after replantation. The pulp was pink, which implies adequate blood supply (patient 2).

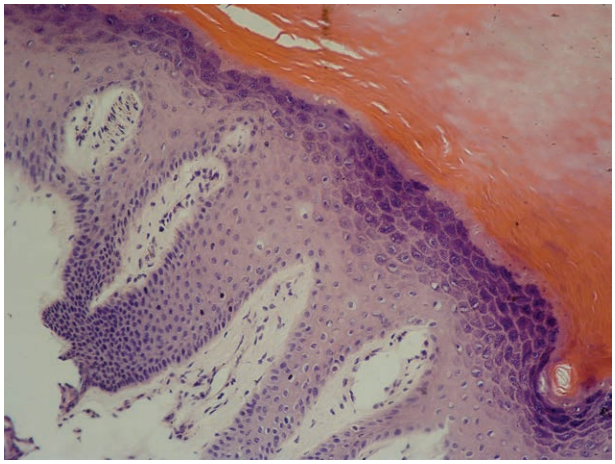

Fig. 4. Hematoxylin and eosin staining of the skin after thawing showed satisfactory skin cell morphology (patient 2).

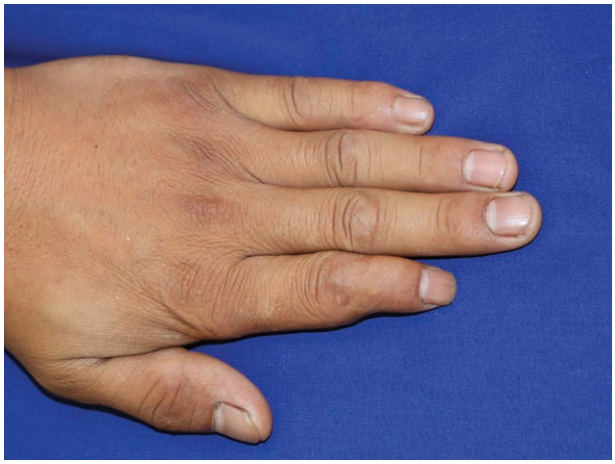

Fig. 5. The dorsal side of the left hand showed mild atrophy of the index finger at the 14-year follow-up visit (patient 2).

At the 6-month follow-up evaluation, mild atrophy of the replanted finger was observed. At the 14-year follow-up visit (Fig. 5), the affected finger demonstrated satisfactory functioning, including grasping and holding actions, and even fine-motor operations such as releasing a knot and pinching a toothpick, in addition to successfully grasping and holding [**see Video (online)**, which shows that the function of the affected finger was favorable]. The patient achieved 0- to 80-degree active flexion at the proximal interphalangeal joint (although radiography showed what seemed like slight hyperextension), and 45- to 60-degree active flexion in the distal interphalangeal joint. Static two-point discrimination was 10 mm, and the Semmes-Weinstein monofilament test result was 2.83. Radiography showed that the bone had healed at the 14-year follow-up visit (Fig. 6).

DISCUSSION

Currently, limbs with complex structures and functions are only stored in solutions at temperatures above 0°C (i.e., using noncryopreservation techniques).[1–5] Nakagawa et al. found that the

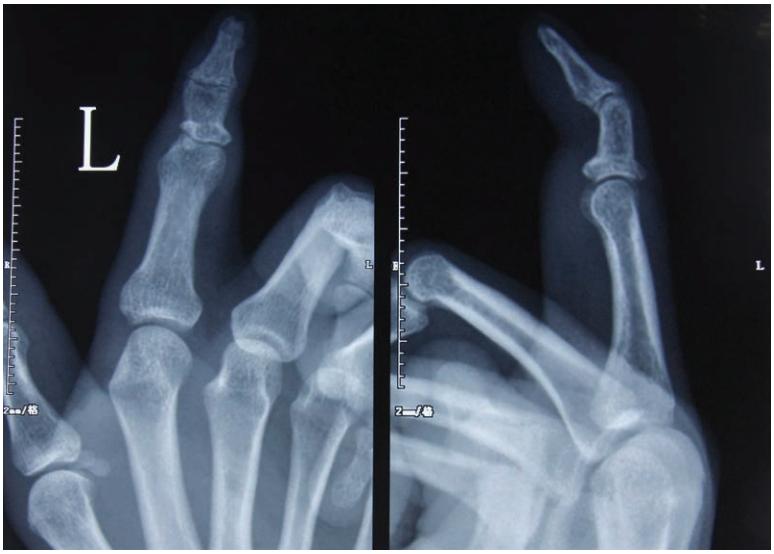

Fig. 6. Radiograph of the index finger at the 14-year follow-up visit (patient 2).

maximum storage time of a limb was 8 hours at −1°C but not at 4°C.[6] In 1995, Kour et al. successfully replanted two amputated upper limbs that had been stored in University of Wisconsin solution at 4°C for 7 and 11.25 hours.[7] Until now, the effective time window within which postconditioning is most effective for the salvage of skeletal muscle was between 4 and 6 hours of ischemia at room temperature.[8,9] Lin et al. reported 14 cases of hand or finger replantation after more than 24 hours. However, such a short time cannot meet clinical needs.[10] In 1949, Polge et al. found that sperm pretreated with glycerol would revive after cryopreservation.[11] Since then, cryopreservation techniques for biological cells and tissues have come into the purview of clinicians. However, these techniques have been limited only to the preservation of biological cells or tissues. Cryopreservation is the best approach for storing specific tissues, including skin grafts, islet cells, and ovaries, which are nonvascularized composite tissues (organs).[12] With regard to intact organs, successful transplantations of other cryopreserved animal or human vascularized composite tissues have not been reported, except for the transplantation of a rat ovary and hind limb. In 2002, Wang et al. reported the successful replantation case of an intact cryopreserved rat ovary in *Nature*.[13] That same year, we reported our first success with rewarming and replanting (by means of vascular anastomosis) a cryopreserved rat hind limb.[14] The two cases we present in this work are the first cases to have undergone successful replantation of cryopreserved human vascularized composite tissues. These successful studies illuminate the cryopreservation of in vitro vascularized composite tissues/organs.

We believe that the current application scope of cryopreservation techniques includes small limbs with minimal amounts of muscle tissue for the following reasons. First, despite the intact cryopreserved rat ovary successfully replanted by Wang's group in 2002 or the cryopreserved amputated rat hind limbs and human fingers replanted by our group, these examples are all small tissues or organs with vascularization. In general, large organs are difficult to revive after cryopreservation because of the permeation limitations of large biological systems and the apparently different biological properties of the various cells or tissues in an organ at cryopreservation.[14–18] Second, myocytes join together in tissues that might be either striated or smooth, depending on the presence or absence, respectively, of the organized, regularly repeated arrangements of myofibrillar contractile proteins called myofilaments. Thus, the structure and function of myocytes are too complicated to endure any problems caused by the process of cryopreservation; therefore, the cryopreservation of muscle tissue is challenging.[19]

In reporting this study, we hope that this technology is recognized by more investigators in the field to further promote the robust development of vascularized composite tissue/organ transplantation technologies. We also believe that the current scope of application of these cryopreservation techniques includes limbs with minimal amounts of muscle tissue.

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