

## Brief Report

## REPLACEMENT OF AN AVULSED PHALANX WITH TISSUE-ENGINEERED BONE

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FOR several decades, various approaches have been used to replace bone lost to trauma and disease. In 1908, Lexer<sup>1</sup> described attempts to reconstruct joints with newly amputated or cadaveric allografts. In more recent years, autografts<sup>2</sup> and allografts<sup>3,4</sup> have been used extensively to replace bone. Several natural or synthetic bone substitutes have also been used alone<sup>5-9</sup> or in conjunction with demineralized bone<sup>10</sup> or autologous bone as vascularized or free (nonvascularized) grafts.<sup>11,12</sup> There have been numerous reports on the use of polypeptides<sup>13,14</sup> or demineralized bone powder<sup>14,15</sup> to stimulate the differentiation of mesenchymal tissue into bone.

More recently, living cells have been implanted in conjunction with inert materials.<sup>16</sup> In this type of tissue engineering, living cells are implanted in a recipient after the cells are seeded in some type of scaffolding or template, which guides tissue regeneration. The template is designed and constructed so that cells can be nourished as they generate a new matrix and as vessel ingrowth occurs and function is restored.

We report the use of a tissue-engineered distal phalanx to replace this bone in a patient who had a partial avulsion of the thumb. The procedure resulted in the functional restoration of a stable and biomechanically sound thumb of normal length, without the pain and complications that are usually associated with harvesting a bone graft.

### CASE REPORT

The patient was a 36-year-old man in whom the dorsal skin, nail and nail bed, extensor tendon, and distal phalanx of the left thumb had been torn off in a machine accident (Fig. 1A). He was right-handed. His medical history was otherwise unremarkable. Within two hours after the injury, the wound was debrided and covered with a pedicle of abdominal skin (Fig. 1B). The pedicle

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was partially dissected from the abdomen on postoperative days 9 and 14, and the flap of skin on the thumb was completely divided from the abdomen on postoperative day 19. The donor site healed well, and the abdominal skin attached well to the thumb (Fig. 1C).

Before the pedicle was dissected from the abdomen, the possibility of generating a tissue-engineered phalanx was discussed with the patient. The patient agreed to this type of procedure, and a plan to undertake it was therefore developed. The institutional human-studies committee approved the plan, and the patient gave written informed consent.

### METHODS

#### Procurement and in Vitro Culture of Autologous Cells

At the time the flap of skin was divided from the abdomen, eight sections of periosteum, each 1 cm<sup>2</sup>, were harvested from the distal part of the left radius by sterile techniques. The excised sections of periosteum were placed in phosphate-buffered saline solution containing antibiotics, transported to the laboratory, and placed into 75-mm tissue-culture flasks containing tissue culture medium (medium 199, Life Technologies, Grand Isle, N.Y.) with 10 percent fetal-calf serum, ascorbic acid (50 mg per milliliter), L-glutamine (292 mg per milliliter), penicillin (100 units per milliliter), streptomycin (100 mg per milliliter), and ergocalciferol (40 ng per milliliter). The flasks were incubated at 37°C in an atmosphere of 5 percent carbon dioxide for the next nine weeks, during which time the culture medium was changed every two to three days. After seven weeks, the fetal-calf serum was replaced by serum from the patient. During the nine-week incubation period, cells shed from the periosteum multiplied to form a monolayer on the bottom of each flask.

After nine weeks, the cells were removed from the flasks with trypsin and counted with a hemacytometer. Viability was determined by staining with trypan blue. A total of 20 million viable cells were obtained.

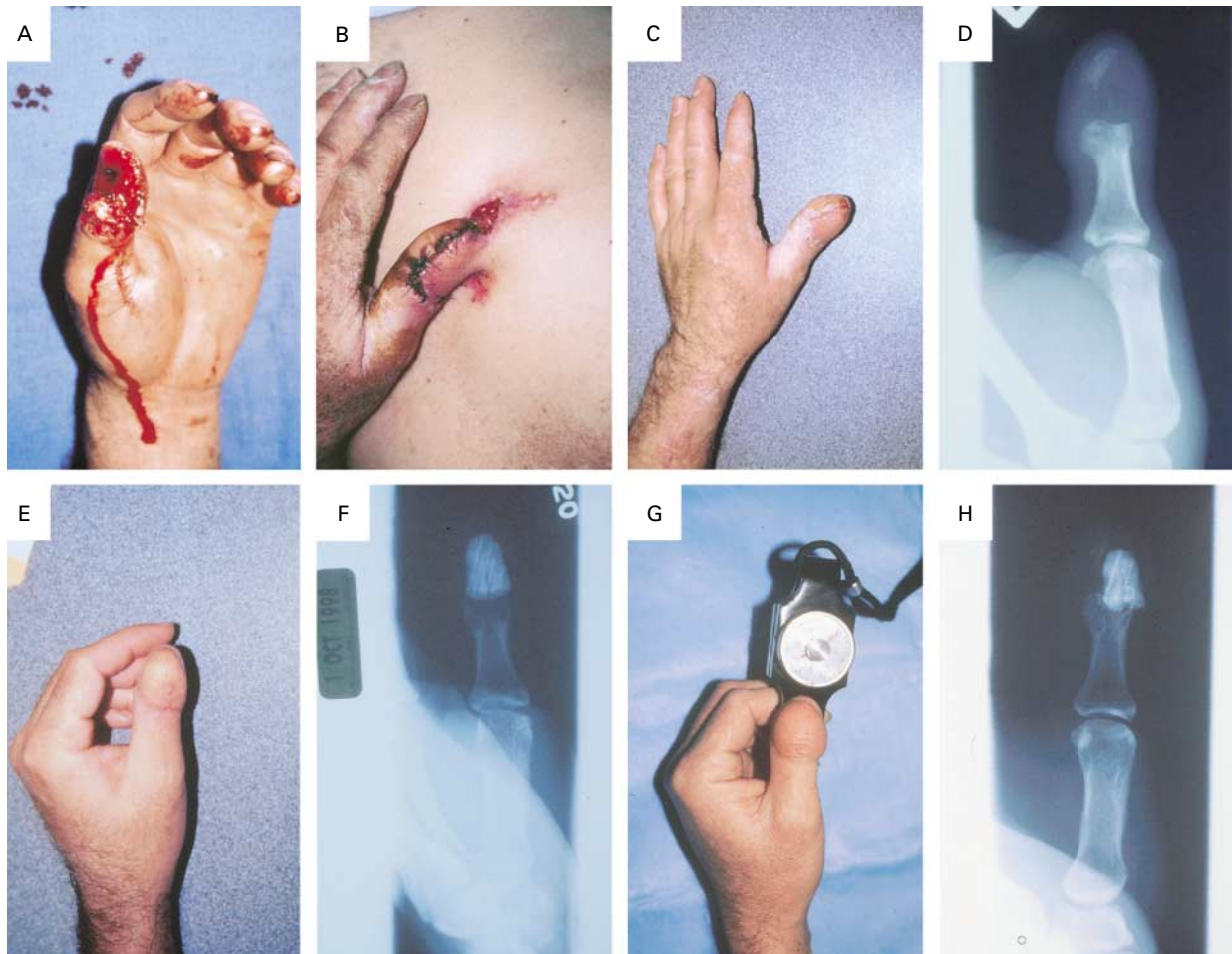
#### Implantation of the Cell-Scaffold Complex

Approximately 12 weeks after the injury, the skin graft on the dorsum of the thumb was incised longitudinally while the patient was under general anesthesia. A pocket was created beneath the flap of the thumb and prepared for placement of the cell-scaffold complex, which was created as follows. Under sterile conditions, a block of specially treated natural coral (porous hydroxyapatite; 500-pore Pro Osteon, Interpore International, Irvine, Calif.) was carved with a motorized burr into the approximate size and shape of the distal phalanx of the right thumb as seen on radiographs. The 20 million periosteal cells were suspended in 1.5 ml of 1 percent alginate acid (Pronova Biopolymer, Oslo, Norway) in saline.

The porous coral implant was placed into the pocket and injected with the cell suspension with an 18-gauge needle. The needle was inserted longitudinally at the apex of the implant and advanced into the center of the implant, and the cells were injected as the needle was slowly withdrawn. The cell suspension was fixed in position by applying 1 ml of a solution of calcium chloride (0.5 mmol per liter) onto the surface of the implant. This step resulted in the formation of a stable calcium alginate hydrogel that encapsulated the cells within the gel that saturated the coral implant. The wound was then irrigated with saline and closed, and the thumb was splinted.

#### Documentation of the Development of Bony Tissue

The thumb remained continuously splinted for eight weeks and intermittently splinted for an additional four weeks, and the patient was followed clinically. A Greenleaf evaluation, which is a standardized method of evaluating functional impairment after a hand injury, according to guidelines published by the American Medical Association,<sup>17</sup> was also performed. With this method, the degree of scar formation, degree of sensation, range of motion, and grip strength are assessed to determine the extent of impairment of the hand, arm, and total body. This evaluation is typically done in a patient with a hand injury when recovery is thought to be maximal.



**Figure 1.** Serial Photographs and Radiographs of the Patient's Thumb after Injury and during Treatment.

Panel A shows the thumb at the time of the patient's presentation to the emergency department, and Panel B the thumb one week after the injury was covered by a pedicle of abdominal skin. Separation of the skin flap from the abdomen and closure of the graft resulted in a thumb of normal length but without a distal phalanx. Six weeks after the injury, the thumb was well healed (Panel C). Panel D shows a radiograph of the thumb before implantation of the coral scaffold seeded with autologous periosteal cells. Six weeks after the implantation, the thumb was of normal length (Panel E), and a radiograph shows that the implant was fairly well aligned with the proximal phalanx (Panel F). Twenty-eight months after the implantation, the patient had good pinch strength (2.3 kg) with the tissue-engineered phalanx (Panel G). A radiograph of the thumb obtained 28 months after the implantation shows that the implant has begun to remodel, with formation of an outer cortex (Panel H).

Radiographs of the thumb were obtained before the implantation and 6 and 28 weeks afterward. The mineral density of the implant was measured 1 week, 5 months, and 10 months after the implantation with a bone densitometer (Hologic QDR-4500A, Waltham, Mass.). A magnetic resonance imaging (MRI) study of the implant was performed six weeks after the implantation. Biopsy specimens of the implant were obtained at 10 months while the patient was under general anesthesia. Some of the biopsy specimens were evaluated microscopically after staining with toluidine blue and trichrome, and some of the specimens were embedded in a polyester resin, coated with gold palladium, and subjected to quantitative histomorphometry by scanning electron microscopy.

### RESULTS

Within 10 days after he received the implant, the patient was able to use his hand fairly well with the

aid of a splint. Three months after the implantation he returned to work as a landscaper. A Greenleaf evaluation performed one year after the implantation revealed 10, 9, and 5 percent impairment of function of the hand, the arm, and the total body, respectively. In contrast, functional impairment of these areas averages 22, 20, and 12 percent, respectively, in patients in whom the distal phalanx of the thumb is amputated.

Twenty-eight months after the implantation, the patient had a thumb of normal length and strength, with some sensation. The tissue-engineered phalanx enabled him to work and perform most daily activities well. The interphalangeal joint became encapsulated

with stable fibrous tissue, which gave him a pinch strength of 2.3 kg (5 lb) (Fig. 1G) and a grip strength of 27.3 kg (60 lb). At the interphalangeal joint, he had no active range of motion and about 15 degrees of passive range of motion. He was able to discriminate two distinct tactile points at least 4 mm apart.

Six weeks after the implantation, MRI examination performed after the injection of gadolinium revealed enhancement of much of the implant, a finding suggestive of vascular perfusion. Radiographs obtained before the implantation and 6 weeks and 28 months afterward showed some dorsal subluxation but no loss of volume or fragmentation of the implant (Fig. 1D, 1F, and 1H). The mineral density of the implant was 0.903 g per cubic centimeter immediately after the implantation and 0.690 and 0.481 g per cubic centimeter at 5 and 10 months, respectively, after the implantation, as compared with 0.382 g per cubic centimeter in the contralateral phalanx.

Gross examination of the implant in situ at the time of biopsy revealed that it was vascularized, non-fragmented, and well incorporated into the surrounding tissue. Light-microscopical examination of the biopsy specimens revealed new bone with a lamellar architecture that was well integrated with the coral scaffold. Quantitative histomorphometric analysis revealed that 30 percent of the volume of the implant was coral, 5 percent was lamellar bone and ossified endochondral tissue, and the remainder was soft tissue and blood vessels.

### DISCUSSION

Successful tissue engineering involves the implantation of living cells with synthetic scaffolding and results in the generation of new tissue. Tissue-engineered bone has many advantages over autologous or cadaveric bone grafts or synthetic materials that are not seeded with cells. Bone autografts that are not anchored to adjacent bone are typically resorbed<sup>18</sup> and thus are not an effective long-term treatment. Furthermore, harvesting of bone autografts often results in donor-site morbidity.<sup>19</sup> Like bone autografts, cadaveric bone grafts are usually resorbed, and they carry the added risk of disease transmission<sup>20</sup> from the donor tissue. Synthetic materials used without cells are also subject to degradation in vivo and can elicit foreign-body reactions or inflammation that is detrimental to the implant and surrounding tissues.<sup>21</sup>

The tissue-engineered bone generated in this patient was created by a process that involved minimal donor-site morbidity, with no risk of disease transmission. Coral (porous hydroxyapatite) was chosen as the material for the scaffold because it is biocompatible and is conducive to bone growth. It also resists deformation,<sup>22</sup> and it can be used to bridge partial bone defects when implanted alone<sup>23-27</sup> or when implanted after being seeded with cells.<sup>28</sup> When coral alone is not in direct contact with bone, no bone

forms within it.<sup>29-31</sup> When it is seeded with marrow cells<sup>30</sup> or with cells derived from the periosteum,<sup>31</sup> however, and placed in subcutaneous tissue that is not adjacent to native bone, new bone forms.

We chose to seed our coral scaffold with periosteal cells because osteoblastic cells shed from the periosteum form bone in animals.<sup>32-35</sup> Alternative sources of cells, such as mesenchymal stem cells<sup>36</sup> or stromal cells of bone marrow, may also prove to be effective. The current case suggests that the use of tissue-engineered bone may be an effective approach to the treatment of bone loss due to trauma or disease.

A U.S. patent entitled "Guided Development and Support of Hydrogel-Cell Compositions" (6027744) was issued on February 22, 2000. Dr. Charles A. Vacanti is one of the inventors. The patent is owned solely by the University of Massachusetts Medical School.

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**CORRECTION**

**Replacement of an Avulsed Phalanx with  
Tissue-Engineered Bone**

Replacement of an Avulsed Phalanx with Tissue-Engineered Bone  
. On page 1511, in the first paragraph of the Methods section, the  
values for ascorbic acid, L-glutamine, and streptomycin should have  
read, "µg per milliliter," not "mg per milliliter," as printed.