

OPEN

Collection of Wound Exudate From Human Digit Tip Amputations Does Not Impair Regenerative Healing

A Randomized Trial

Tobias Kisch, MD, Julia Maria Klemens, MSc, Katharina Hofmann, MSc, Eirini Liodaki, MD, Matthias Gierloff, MD, Dirk Moellmeier, MD, Felix Stang, MD, Peter Mailaender, MD, PhD, Jens Habermann, MD, PhD, and Matthias Brandenburger, PhD

Abstract: The regrowth of amputated digit tips represents a unique regenerative healing in mammals with subcutaneous volume regrowth, restoration of dactylogram, and suppression of scar formation. Although factor analysis in amphibians and even in mice is easy to obtain, safety of harvesting biomaterial from human digit tip amputations for analysis has not yet been described.

The aim of this study was to evaluate if recovering wound exudate does hamper clinical outcome or influence microbiologic or inflammation status.

A predefined cohort of 18 patients with fresh digit tip amputations was randomly assigned to receive standard therapy (debridement, occlusive dressing) with (n=9) or without (n=9) collection of the whole wound exudate in every dressing change. Primary endpoint (lengthening) and secondary endpoints (regeneration of dactylogram, nail bed and bone healing, time to complete wound closure, scar formation, 2-point discrimination, microbiologic analysis, inflammatory factors interleukin (IL)-1 α , tumor necrosis factor- α , IL-4, and IL-6) were determined by an independent, blinded observer.

Patients' characteristics showed no significant differences between the groups. All patients completed the study to the end of 3 months follow-up. Exudate collection did not influence primary and secondary endpoints. Furthermore, positive microbiologic findings as well as pus and necrosis-like appearance neither impaired tissue restoration nor influenced inflammatory factor release.

Here, the authors developed an easy and safe protocol for harvesting

wound exudate from human digit tip amputations. For the first time, it was shown that harvesting does not impair regenerative healing. Using this method, further studies can be conducted to analyze regeneration associated factors in the human digit tip.

DRKS.de Identifier: DRKS00006882 (UTN: U1111-1166-5723).

(*Medicine* 94(41):e1764)

Abbreviations: BMP = bone morphogenetic protein, Cdkn = cyclin-dependent kinase inhibitor, DIP = distal interphalangeal joint, IL = interleukin, MRL = murphy roths large, NIDDM = noninsulin-dependent diabetes mellitus, NSC = nail stem cell, PBS = phosphate-buffered saline, rcf = relative centrifugal force, SCA = stem cell antigen, SDF = stromal cell-derived factor, TNF = tumor necrosis factor.

INTRODUCTION

Amputated digit tips represent a unique regenerative capability in mammals.¹⁻³ Success of regeneration depends on the amputation level.⁴ Although wounds in mammals usually heal in a reparative manner with wound site contraction and scar formation, digit tips reveal a nearly perfect healing of nail bed, bone, and subcutaneous tissue. Furthermore, volume of subcutaneous tissue increases and dermal ridges including dactylogram renew. This phenomenon is significantly pronounced in children, but it is also present in older and even in chronically ill patients. In comparison to conservative treatment, surgical interventions on digit tips generally lead to an impaired sensibility and the formation of scar tissue in humans.^{5,6} The use of occlusive dressings is beneficial because of a wet environment, which supports the regeneration process as an optimal milieu.⁷

Etiology of this unique healing quality in digit tips is still unknown. The mouse digit tip model was described to imitate human digit tip regeneration very well and is therefore frequently used for loss-of-function and gain-of-function studies.⁸ These studies imply that bone morphogenetic protein (BMP) and stromal cell-derived factor-1 α signaling might be an essential part of the endogenous regeneration response.^{9,10} Yu et al^{11,12} showed that treatment with either BMP-2 or BMP-7 induced regeneration of proximal digit tip injuries, whereas Wu et al¹³ and Lynch et al¹⁴ mentioned a lack of migratory response in cells from the proximal compared with the distal part. Furthermore, nail stem cells from the proximal nail matrix have been shown to increase digit tip regeneration by Wnt pathway activation.¹⁵ Moreover, regeneration of bone and nail defects might be influenced by nerve-derived factors.¹⁶ On the contrary, regrowth of amputated toe tips in rats was not interrupted by denervation, but healing was delayed.¹⁷ In an in vitro human fetal model, transcription repressor Mx1 was identified

Editor: Mahmood S Choudhery.

Received: July 29, 2015; revised: September 13, 2015; accepted: September 14, 2015.

From the Department of Plastic Surgery and Hand Surgery, University Hospital Schleswig-Holstein Campus Lübeck, University of Lübeck (TK, EL, MG, DM, FS, PM); Fraunhofer Research Institution for Marine Biotechnology EMB (JMK, KH, MB); and Department of Surgery, Section for Translational Surgical Oncology and Biobanking, University Hospital Schleswig-Holstein Campus Lübeck, University of Lübeck, Lübeck, Germany (JH).

Correspondence: Tobias Kisch, MD, Department of Plastic Surgery and Hand Surgery, University Hospital Schleswig-Holstein Campus Lübeck, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany (e-mail: tobias.kisch@uksh.de).

This study was funded by the University of Lübeck (J20-2015).

Meetings at which the article has been presented:

11. Sept. 2014 Congress of the German Society of Plastic, Reconstructive and Aesthetic Surgery, Munich, Germany.

26. June 2014 Regenerative Medicine Symposium, Lübeck, Germany.

The authors have no conflicts of interest to disclose.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

This is an open access article distributed under the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0, where it is permissible to download, share and reproduce the work in any medium, provided it is properly cited. The work cannot be changed in any way or used commercially.

ISSN: 0025-7974

DOI: 10.1097/MD.0000000000001764

to be of special interest in the regrowing fingertip,^{18,19} but does not represent a marker for a pluripotent stem cell population.²⁰ The stem cell marker stem cell antigen-1, however, was expressed in adult mice digit tip regeneration.²¹ Moreover, regeneration might be linked to an absence of p53-dependent p21 cyclin-dependent kinase inhibitor protein as described in MRL-mice and *cdkn1a^{tm1/Tyj/J} p21^{-/-}* mice.²²

Because the ability to regenerate tissues is known to differ strongly between fetal and adult mice and even between different mice strains,²³ direct transfer of these findings from mice to human might be highly speculative. Therefore, regeneration processes after adult human digit tip amputations need to be further examined. Limb regeneration in amphibians and digit tip regeneration in mice have recently been shown to be based on local environment rather than on systemic factors.^{24,25} Therefore, the first aim should be to address the local wound milieu in humans.

The current randomized trial was conducted to determine feasibility and clinical safety of collecting the gel-like wound exudate from fresh human digit tips after amputation.

METHODS

This report complies with the reporting standards established by the revised Consolidated Standards of Reporting Trials consensus statement. The study was registered at DRKS.de (ID:DRKS00006882) with the UTM: U1111-1166-5723.

Participants

A prospective randomized trial was conducted from October 2014 to March 2015, which was approved by the Institutional Ethics Committee at the University of Lübeck, under authorization number 13-041. During the period, 18 patients with fresh and sharp digit tip amputations were enrolled in the study. Eligibility criteria were male and female patients aged ≥ 18 years who could give their informed consent. Patients with an amputation of less than 6-mm length (wound to distal interphalangeal joint flexion crease) or inclusive lunula, dirty wounds, or immunosuppressive medication were excluded. Smokers and patients suffering from noninsulin-dependent diabetes mellitus were included in this study. The research was carried out in accordance with the Declaration of Helsinki. Once eligibility was confirmed, study patients were assigned randomly to study or control group.

Sample Size and Randomization

Sample size was prospectively set at 9 patients per group to detect a shortening in mean length of 3- and a 2-mm standard deviation with a power of 80% and a type I error of 0.05 in a 2-sided test. Patients were randomized (1:1 ratio) to undergo digit tip amputation therapy in accordance with institutional standards specified below or the same treatment with collection of the whole wound exudate every dressing change. A computerized random number generation (without stratification) was used for randomization. The sequence was concealed to study group assignment. The primary endpoint and the secondary endpoints were determined by an independent, blinded observer.

Primary and Secondary Outcome

The primary outcome variable was distal phalanx lengthening of amputated digit tips. The study participants who have provided informed consent to participate in this study

were followed in outpatient clinic weekly to complete wound healing. Complete wound healing was defined as a full re-epithelialization of the digit tip. Distal phalanx lengthening was measured and compared with initial length and contralateral distal phalanx. Microbiologic analysis was performed using smears. After 3 months follow-up, dactylogram and scar size were evaluated by fingerprint and 2-point discrimination was compared with the opposite finger.

Treatment in the Study Group and the Control Group

Directly after trauma, the outpatients received an antiseptic wound bath (Serasept®, Serag-Wiessner, Naila, Germany) and a surgical debridement of the digit tip wound. Distal phalanx length and wound size were measured with a caliper; a microbiologic smear was performed as well as photo documentation. An occlusive dressing (Tegaderm™, 3M, St. Paul, MN) was applied to the wound, protected by a cotton bandage. On day 1, 5, and afterward weekly, a smear and an antiseptic wound bath were performed. Wounds were examined to ensure clinical and microbiologic safety and a new occlusive dressing was applied. Occlusive dressings were continued to the day of complete epithelialization. Then 2-point discrimination was tested with a Sensidisc® (Sensidisk, Hannover, Germany). Moreover, distal phalanx length measuring, scar size evaluation by fingerprint, and photo documentation of the digit tip were repeated.

Harvesting and Analyzing the Wound Exudate

In experimental group, the whole gel-like wound exudate was collected by abrasion with a sterile scalpel on day 1, day 5, and afterward weekly after trauma, whereas in control group, the exudate was left undisturbed. Biomaterial was collected in a small prechilled tube. After weighing, it was diluted in phosphate-buffered saline (1:10) and centrifuged at 2000 relative centrifugal force for 10 minutes at 4 °C. Supernatant was then transferred to nitrogen in 100 μ L samples. Time from harvesting to freezing was kept under 30 minutes. For further experiments, samples were thawed on ice. Quantitative cytokine analysis was performed using a Q-Plex™ ELISA (Quansys Biosciences, Logan, UT) in accordance with the manufacturers' description. Briefly, samples were loaded to the array plate containing immobilized antibodies spotted and blocked by the manufacturer. Antigen standards were loaded for an 8-point standard curve. After incubation, immobilized cytokines were labeled with biotinylated antibodies and then incubated with streptavidin-conjugated horse radish peroxidase. Antibodies and peroxidase were part of the Q-Plex™ ELISA kit. Directly after addition of the substrates, chemiluminescence was detected with the Fusion SL (Vilber Lourmat, Fisher Biotech, Australia). Signals were evaluated via Q-View™ software (Quansys Biosciences, Logan, UT).

Statistical Analysis

SigmaPlot™ Version 12.3 (Systat, San José, CA) was used for statistical analysis. Categorical variables between the groups were compared using a 2-sided Fisher exact test. Continuous data are given as mean \pm standard deviation. Normal distribution was tested using the Shapiro-Wilk test. Then, data were compared using an unpaired *t* test or the Mann-Whitney *U* test. For continuous data in 1 patient, the paired *t* test or Wilcoxon rank sum test was used. A 2-sided *P*-value less than 0.05 was considered significant.

TABLE 1. Distribution of Study Patient Characteristics

Characteristic	Experimental Group n = 9	Control Group n = 9
Age (years), mean ± SD	39.0 ± 15.8	41.8 ± 18.3
Sex (male), n (%)	8 (89%)	8 (89%)
NIDDM, n (%)	2 (22%)	1 (11%)
Smokers, n (%)	3 (33%)	2 (22%)
Damaged nail bed, n (%)	5 (55%)	3 (33%)
Damaged bone, n (%)	3 (33%)	3 (33%)
Wound size (qmm), mean ± SD	103.7 ± 49.7	94.5 ± 56.0
Loss of length (mm), mean ± SD	7.6 ± 1.9	7.8 ± 2.0

Patients' characteristics showed no significant differences between the groups. NIDDM = noninsulin-dependent diabetes mellitus, qmm = square millimeters, SD = standard deviation.

RESULTS

Patients

All patients completed the study without exclusion, loss to follow-up or incomplete data. Baseline characteristics, especially age ($P = 0.735$), wound size ($P = 0.718$), and loss of length ($P = 0.810$), showed no significant differences between experimental and control group (Table 1). We observed high compliance in both groups. Most patients had injured their left hand.

Collection of Wound Exudate

Collection of the gel-like exudate was easy to obtain. The patients tolerated material harvesting without pain, but 1 experienced syncope. All patients described a sensation of pricking while abrasion was performed with the scalpel. To prevent contamination with fresh blood, cutting was avoided. Most biomaterial could be harvested on day 1 and day 5 posttrauma (5.6 ± 1.2 and 6.1 ± 2.9 samples) because of a weight of biomaterial of $51.7 \text{ mg} \pm 10.4$ on day 1 and $92.6 \text{ mg} \pm 42.0$ on day 5 after trauma. Because the wounds were dry in most patients, 2 or 3 weeks after trauma, wound exudate was not further suitable for collection.

Primary and Secondary Outcome Assessment

All amputated digit tips healed with a mean lengthening of $6.6 \text{ mm} \pm 1.2$. Mean lengthening for patients who did and did not undergo collection of the wound exudate was $6.6 \text{ mm} \pm 1.0$ and $6.7 \text{ mm} \pm 1.4$, respectively ($P = 0.850$) (Fig. 1). In subgroup analysis, diabetic patients and smokers did not show significant differences to nondiabetic patients ($P = 0.264$) or nonsmokers ($P = 0.381$). Every digit wound resulted in nearly perfect healing with an increase of subcutaneous fat and bone (Fig. 2). Dactylogram was regenerated in all patients without significant scar tissue formation. Scar area in experimental group was $1.1 \text{ qmm} \pm 1.2$ versus $1.2 \text{ qmm} \pm 1.1$ in control group ($P = 0.878$). Wound closure was complete within 5 to 8 weeks (Fig. 3). In case of nail bed defects, damaged structures showed flawless healing (Fig. 4A and B). In experimental group, 8 microbiologic smears showed gram-positive (*Staphylococcus*,

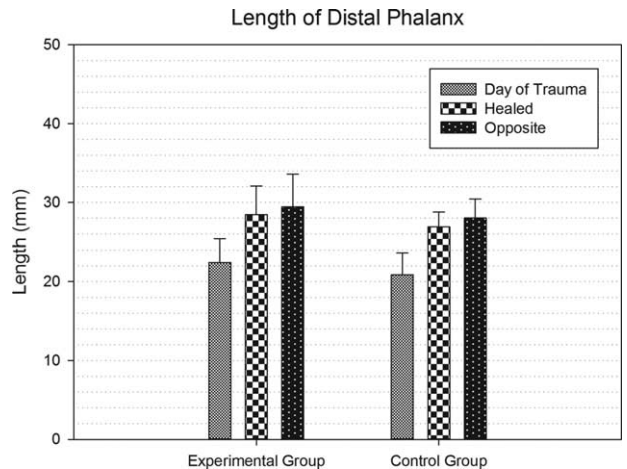


FIGURE 1. Length of distal phalanges measured from distal tip to distal interphalangeal joint flexion crease. An increase of subcutaneous structures significantly fills the tissue defect to the day of complete healing ($P < 0.001$). In comparison to the contralateral distal phalanges, an insignificant shortening after healing is displayed.

Bacillus) and 2 smears showed gram-negative microbes (*Pseudomonas* and *Acinetobacter*). The healing zones regularly produced an unpleasant smell and exudate showed a pus- or necrosis-like appearance (Fig. 4C and D). Inflammatory cytokines, however, did not reveal prodigious infection (Fig. 5). Therefore, occlusive dressing was continued.

In the follow-up, 2-point discrimination did not significantly differ between the wounded and the contralateral fingers in the experimental group ($2.6 \text{ mm} \pm 0.4$ versus $2.6 \text{ mm} \pm 0.4$, $P = 0.760$) and the control group ($2.5 \text{ mm} \pm 0.5$ versus $2.5 \text{ mm} \pm 0.3$, $P = 0.641$). One month after the injury, nearly all of the patients complained about hypersensitivity and some of them realized pain when the healed fingertip was touched. Two months later, pain was no longer reported.

DISCUSSION

In this study, we demonstrated that harvesting wound exudate from fresh human digit tip amputations, and therefore removing parts of the local healing environment, did not impair the regenerative healing capability of the digit tip.

Lassere et al²⁶ detected angiogenesis-promoting factors in digit tip amputations, but the harvesting technique was not elucidated. Different methods of collecting local wound exudate have been described for other regions of the body.²⁷ An easy method is the collection by drainage^{28,29} or just by means of puncture through film dressings or wound blisters with a syringe.^{30,31} Several new methods such as wound blotting by attaching a membrane onto the wound surface allow noninvasive collection for further analysis.³² For use of these methods, wound exudate has to be fluid. In chronic wounds, the collection of debris with a Vidal curette seems to be useful.³³ Each of these techniques was suitable for the detection of cytokines or wound-healing mediators. In the case of digit tip injuries, the defect is covered with a gel-like exudate. Therefore, the optimal exudate collection is by abrasion. Owing to the direct contact of the exudate to the wound bed, the wound environment is closely reflected by the harvested biomaterial.³⁴ The technique described is simple to perform, compatible with standard wound

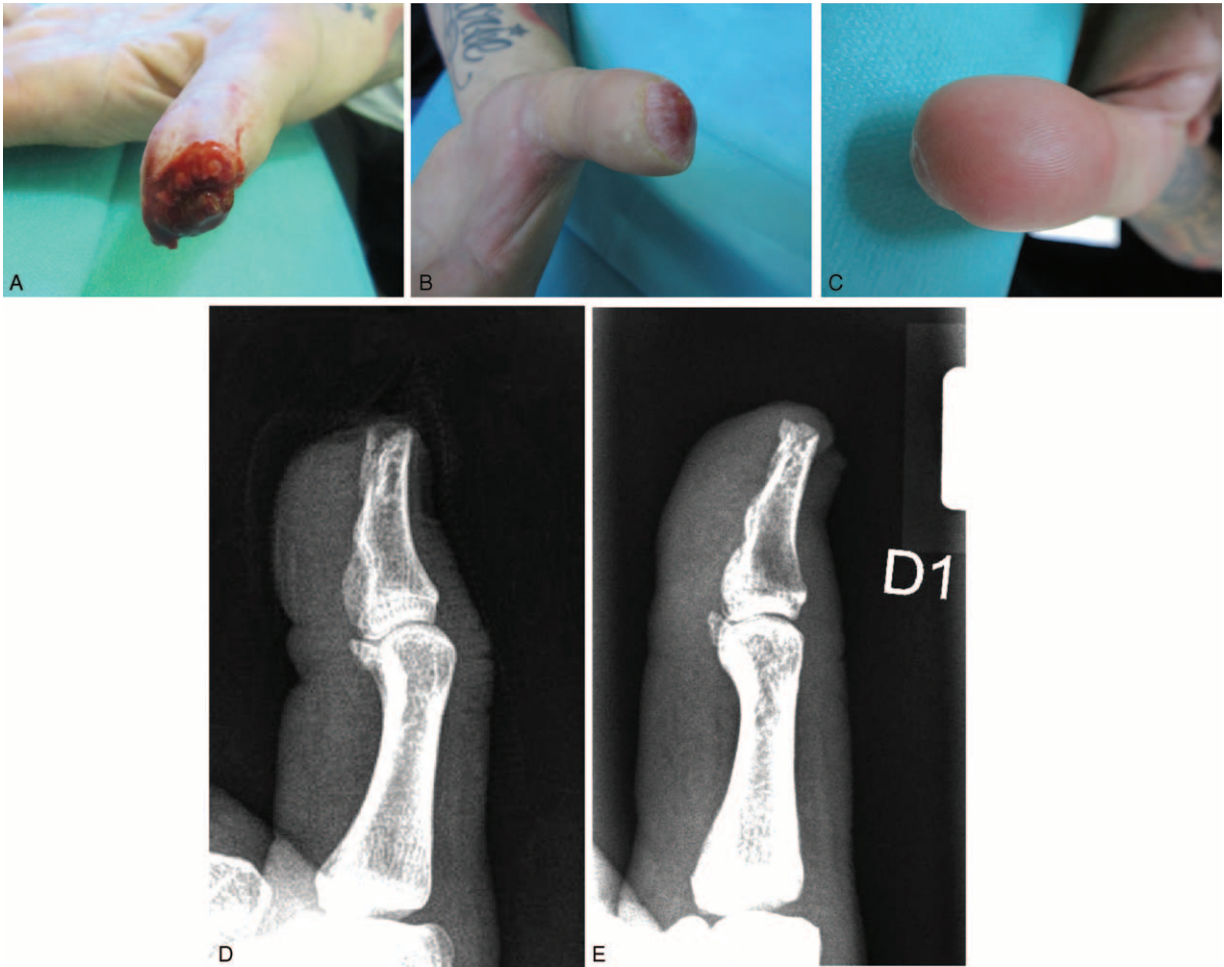


FIGURE 2. Bone and fat defect directly after trauma (A). Increasing volume of subcutaneous fat 3 weeks after trauma (B) and regeneration of dactylogram 3 months after trauma (C) in a diabetic patient from the experimental group. Radiograph in this patient on trauma day (D) and 3 months later (E) showing good soft-tissue coverage of the bone.

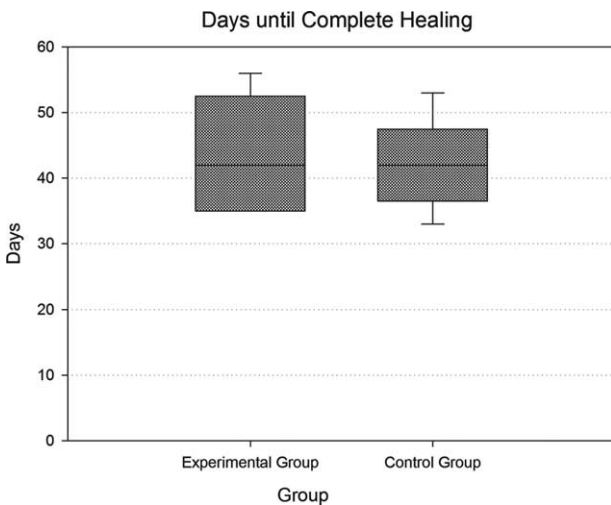


FIGURE 3. Days to complete healing. Collection of the whole wound exudate did not significantly alter the period to complete healing.

care of digit tip amputations and allows direct comparison with wounds in other regions of the body.

Cryopreservation protocol needs to be followed in a standardized manner to prevent preanalytical bias; freeze-thawing cycles should be avoided. In addition, long-term storage of wound exudate in -80°C should be avoided because of potential degradation of cytokines and chemokines.³⁵ Therefore, we recommend storing samples in nitrogen below -150°C .

Inflammatory cytokines, such as interleukin (IL)-1 α , tumor necrosis factor- α , IL-4, and IL-6 were detected in other acute wounds, too, using different harvesting techniques.³⁶ An important point when analyzing wound exudate of acute wounds is addressing the dynamic behavior of cytokines. Detection should therefore be performed at different time points during the wound-healing process. We did not find a relevant increase in inflammatory cytokines, but an elevation in comparison to standard blood values was evident.^{37,38} Nevertheless, we were concerned about the critical contamination by bacteria not representing the normal microbiome. This phenomenon has been described before.²⁶ Usually, the application of occlusive dressings is not recommended in case of infection. Because we did not find a significant increase in the infection parameters,

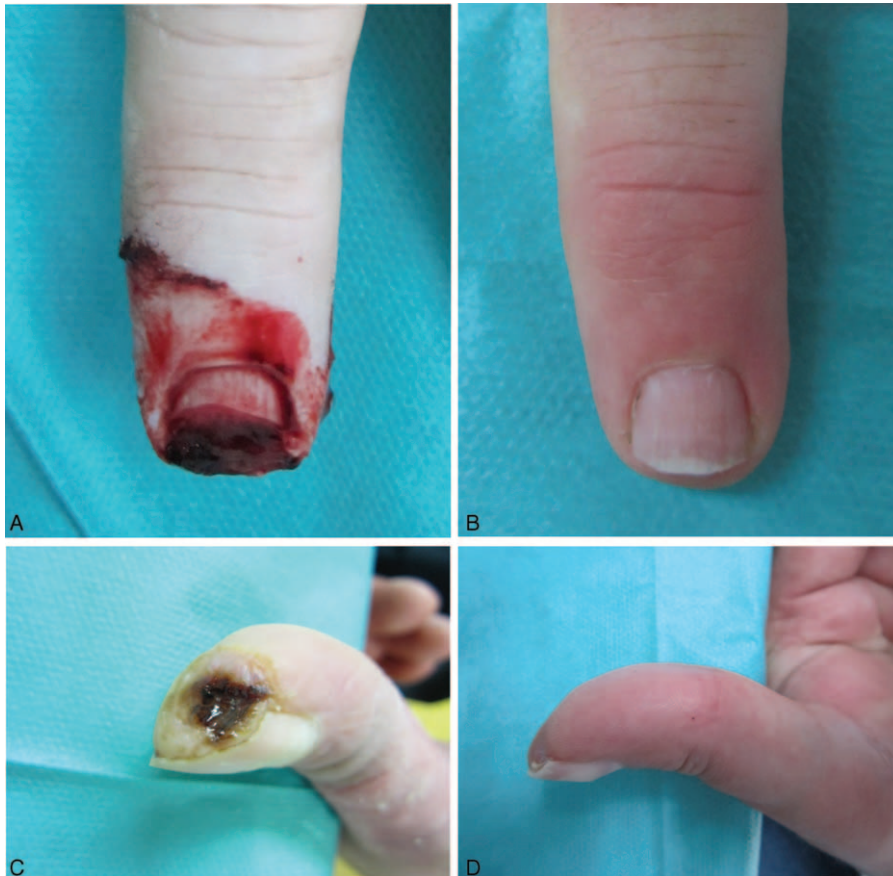


FIGURE 4. Nail bed destruction 1 day after trauma (A) and healing situation 3 months later in a patient from the experimental group (B). Dark zone appears like necrosis 3 weeks after trauma (C), but heals well under occlusive dressing even after harvesting the wound exudate; here 2 months after injury (D).

the treatment protocol was continued without modification. Each wound produced an unpleasant smell. Wound exudate in some cases looked like pus or necrosis. Continuing the use of occlusive dressings after an antiseptic wound bath led to a

regenerative healing as mentioned before. Wound contraction was not measured, but might not have a relevant impact on the healing process in digit tips, because the tissue laxity is very low. As described by other authors, healed digit tips, however, are a bit smaller or thinner than the original. This fact is also known in limb regeneration in amphibians.²⁴

High patient compliance was achieved because a conservative dressing regime was preferred to an operative procedure. Some patients even went to work with the finger dressing. Regarding the time period to complete healing was achieved, our findings are in accordance with those described in mice, emphasizing that the slow healing in fingertips differs greatly from other skin wounds, where rapid wound closure may be associated with the formation of scar tissue.¹¹ Furthermore, 2-point discrimination was at a nearly perfect sensibility as described by other authors.³⁹

The aggregate of proliferating cells involved in the regeneration process is sometimes termed “mammalian blastema”.¹¹ We found that wound exudate of human fingertip amputations appears in a gel-like fashion. There, however, are great differences between a real blastema structure and wound regions in mammalia.⁴⁰

In conclusion, we have developed an easy method to harvest wound exudate from human digit tip amputations. Low variations between the samples indicate that the technique is suitable for quantitative detection of cytokines. Material

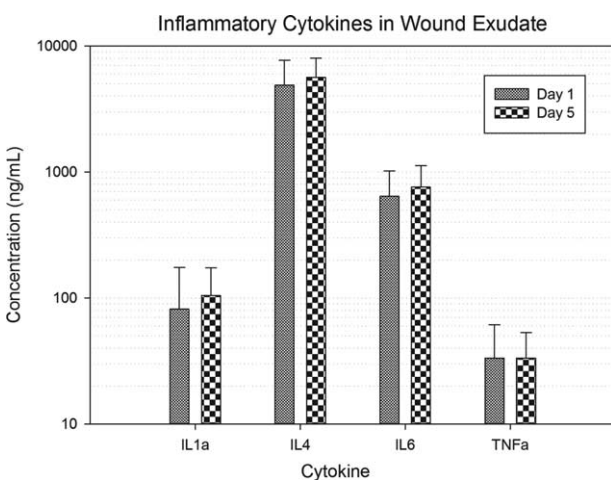


FIGURE 5. Cytokines showed no significant dynamic from day 1 to day 5 posttrauma. Proinflammatory cytokines did not increase.

harvesting did not have a negative impact on the regenerative healing capability. In accordance with the clinical outcome, this is a safe protocol. Hence, further studies need to be performed to analyze regeneration-associated factors and compare digit tip wound healing to other localizations in the human body. In addition, species-specific differences should be sought.

ACKNOWLEDGMENTS

The authors thank T. Gemoll and M. Oberländer for their thoughts and useful discussions, and R. Kaatz for her technical advice. This work is part of the ICB-L (Interdisciplinary Center for Biobanking-Lübeck) and the Innovation Centre for Clinical Cell Technology.

REFERENCES

- Douglas BS. Conservative management of guillotine amputations of the finger in children. *J Paediatr Child Health*. 1972;8:86–89.
- Borgens RB. Mice regrow the tips of their foretoes. *Science*. 1982;217:747–750.
- Singer M, Weckesser EC, Géraudie J, et al. Open finger tip healing and replacement after distal amputation in rhesus monkey with comparison to limb regeneration in lower vertebrates. *Anat Embryol*. 1987;177:29–36.
- Han M, Yang X, Lee J, et al. Development and regeneration of the neonatal digit tip in mice. *Dev Biol*. 2008;315:125–135.
- Das SK, Brown HG. Management of lost finger tips in children. *Hand*. 1978;10:16–27.
- Illingworth CM. Trapped fingers and amputated finger tips in children. *J Pediatr Surg*. 1974;9:853–858.
- Vogt PM, Andree C, Breuing K, et al. Dry, moist, and wet skin wound repair. *Ann Plast Surg*. 1995;34:493–499.
- Simkin J, Han M, Yu L, et al. The mouse digit tip: from wound healing to regeneration. *Methods Mol Biol*. 2013;1037:419–435.
- Lynch KM, Ahsan T. Correlating the effects of bone morphogenic protein to secreted soluble factors from fibroblasts and mesenchymal stem cells in regulating regenerative processes in vitro. *Tissue Eng Part A*. 2014;20:3122–3129.
- Lee J, Marrero L, Yu L, et al. SDF-1/CXCR4 signaling mediates digit tip regeneration promoted by BMP-2. *Dev Biol*. 2013;382:98–109.
- Yu L, Han M, Yan M, et al. BMP signaling induces digit regeneration in neonatal mice. *Development*. 2010;137:551–559.
- Yu L, Han M, Yan M, et al. BMP2 induces segment-specific skeletal regeneration from digit and limb amputations by establishing a new endochondral ossification center. *Dev Biol*. 2012;372:263–273.
- Wu Y, Wang K, Karapetyan A, et al. Connective tissue fibroblast properties are position-dependent during mouse digit tip regeneration. *PLoS One*. 2013;8:e54764.
- Lynch KM, Ahsan T. Modulating the physical microenvironment to study regenerative processes in vitro using cells from mouse phalangeal elements. *Tissue Eng Part A*. 2013;19:1406–1415.
- Takeo M, Chou WC, Sun Q, et al. Wnt activation in nail epithelium couples nail growth to digit regeneration. *Nature*. 2013;499:228–232.
- Rinkevich Y, Montoro DT, Muhonen E, et al. Clonal analysis reveals nerve-dependent and independent roles on mammalian hind limb tissue maintenance and regeneration. *Proc Natl Acad Sci U S A*. 2014;111:9846–9851.
- Mohammad KS, Neufeld DA. Denervation retards but does not prevent toetip regeneration. *Wound Repair Regen*. 2000;8:277–281.
- Allan CH, Fleckman P, Fernandes RJ, et al. Tissue response and Msx1 expression after human fetal digit tip amputation in vitro. *Wound Repair Regen*. 2006;14:398–404.
- Reginelli AD, Wang YQ, Sassoon D, et al. Digit tip regeneration correlates with regions of Msx1 (Hox 7) expression in fetal and newborn mice. *Development*. 1995;121:1065–1076.
- Lehoczky JA, Robert B, Tabin CJ. Mouse digit tip regeneration is mediated by fate-restricted progenitor cells. *Proc Natl Acad Sci U S A*. 2011;108:20609–20614.
- Fernando WA, Leininger E, Simkin J, et al. Wound healing and blastema formation in regenerating digit tips of adult mice. *Dev Biol*. 2011;350:301–310.
- Bedelbaeva K, Snyder A, Gourevitch D, et al. Lack of p21 expression links cell cycle control and appendage regeneration in mice. *Proc Natl Acad Sci U S A*. 2010;107:5845–5850.
- Chadwick RB, Bu L, Yu H, et al. Digit tip regrowth and differential gene expression in MRL/Mpj, DBA/2, and C57BL/6 mice. *Wound Repair Regen*. 2007;15:275–284.
- Kragl M, Knapp D, Nacu E, et al. Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature*. 2009;460:60–65.
- Rinkevich Y, Lindau P, Ueno H, et al. Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. *Nature*. 2011;476:409–413.
- Lasserre G, Bakkouch S, Pauchot J, et al. Reconstruction pulpaire par pansement occlusif: évaluation clinique et analyse biologique du contenu du pansement. *Chir Main*. 2010;29:315–320.
- Löffler MW, Schuster H, Bühler S, et al. Wound fluid in diabetic foot ulceration: more than just an undefined soup? *Int J Low Extrem Wounds*. 2013;12:113–129.
- Nissen NN, Polverini PJ, Koch AE, et al. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol*. 1998;152:1445–1452.
- Kainulainen V. Syndecans, heparan sulfate proteoglycans, maintain the proteolytic balance of acute wound fluids. *J Biol Chem*. 1998;273:11563–11569.
- Ono I, Gunji H, Zhang JZ, et al. Studies on cytokines related to wound healing in donor site wound fluid. *J Dermatol Sci*. 1995;10:241–245.
- He Y, Young PK, Grinnell F. Identification of proteinase 3 as the major caseinolytic activity in acute human wound fluid. *J Invest Dermatol*. 1998;110:67–71.
- Minematsu T, Nakagami G, Yamamoto Y, et al. Wound blotting: a convenient biochemical assessment tool for protein components in exudate of chronic wounds. *Wound Repair Regen*. 2013;21:329–334.
- Kroeze KL, Vink L, de Boer, et al. Simple wound exudate collection method identifies bioactive cytokines and chemokines in (arterio) venous ulcers. *Wound Repair Regen*. 2012;20:294–303.
- Yager DR, Kulina RA, Gilman LA. Wound fluids: a window into the wound environment? *Int J Low Extrem Wounds*. 2007;6:262–272.
- Jager w de, Bourcier K, Rijkers GT, et al. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol*. 2009;10:52.
- Grimstad Ø, Sandanger Ø, Ryan L, et al. Cellular sources and inducers of cytokines present in acute wound fluid. *Wound Repair Regen*. 2011;19:337–347.
- Mooradian AD, Reed RL, Meredith KE, et al. Serum levels of tumor necrosis factor and IL-1 alpha and IL-1 beta in diabetic patients. *Diabetes Care*. 1991;14:63–65.
- Arican O, Aral M, Sasmaz S, et al. Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm*. 2005;2005:273–279.
- Mennen U, Wiese A. Fingertip injuries management with semi-occlusive dressing. *J Hand Surg Br*. 1993;18:416–422.
- Tornini VA, Poss KD. Keeping at arm's length during regeneration. *Dev Cell*. 2014;29:139–145.