Topical collagen powder as a wound healing adjuvant for acute full-thickness punch biopsy-induced human wounds: a pilot study

STUDY PROTOCOL

GWU IRB # 121745

PRINCIPAL INVESTIGATOR:

Adam Friedman, MD
Associate Professor of Dermatology
George Washington University

Medical Faculty Associates
2150 Pennsylvania Avenue, NW
Washington, DC 20037
(202) 741-2625

TABLE OF CONTENTS

1. STATEMENT OF HYPOTHESIS AND OBJECTIVES:

Surgical procedures are an essential piece of a dermatologist's practice. In fact, it has been reported that dermatologists perform over 25 million minor surgical procedures annually. A major focus of these procedures is to restore the integrity of the skin while minimizing scarring and potential complications. With dermatologic surgeries being so numerous, there is much discussion about how to optimize post-surgical wound care while decreasing potential barriers to healing. Recent advances in wound therapy have resulted in the development of advanced wound care therapies, such as collagen-based dressings, for use as adjunctive therapies to combat the molecular defects present in wounds.

We have designed a pilot study investigating the efficacy of Nuvagen™ (collagen powder) in treating skin punch biopsy wounds. The objectives of the study are as follows:

Primary objective:

1. To evaluate the effect of Nuvagen™ (collagen powder) on complete wound healing using punch biopsy sites as an acute full-thickness human wound model based on clinical wound closure.
2. To evaluate the effect of Nuvagen™ (collagen powder) on complete wound healing using punch biopsy sites as an acute full-thickness human wound model based on histology of wound site at 4 weeks following injury.

2. BACKGROUND, SIGNIFICANCE, AND RATIONALE

Wound healing is complex process which requires the restoration of hemostasis, inflammation, proliferation, and remodeling, all of which work in concert to repair the damaged skin barrier in acute wounds.[1,2] Recent advances in wound therapy have resulted in the development of advanced wound care therapies, such as collagen-based dressings, for use as adjunctive therapies to combat the molecular defects present in wounds.

Collagen is a naturally occurring protein that comprises three-quarters of the weight of human skin.[3] Fibroblasts synthesize collagen molecules, which are composed of three polypeptide chains that together form a triple-helical structure, allowing covalent cross-linking and formation of larger fibrils and fibers.[4] Collagen's unique structure makes it an essential component of the extracellular matrix (ECM), providing remarkable tensile strength and structural support to skin and other connective tissues.[4–6] During normal wound healing, collagen acts as a scaffold for cellular ingrowth and organized collagen deposition.[5,7] In addition, proteases that degrade native collagen release polypeptide fragments that act as chemotactic molecules to recruit inflammatory mediators, promote keratinocyte migration, and stimulate the proliferation of fibroblasts and subsequent collagen deposition.[5,7,8]

Powdered or fractionated collagen products are developed through a proprietary process that involves the breakdown of natural collagen into smaller components.[5,7,9] These collagen particles exhibit minimal cross-linking and are hypothesized to quickly replace degraded collagen fragments by bypassing enzymatic breakdown within the wound environment. Furthermore,

powdered collagen can act as a signaling molecule directly upon application, recruiting inflammatory cells and fibroblasts and supporting keratinocyte migration and ECM production.[10,11]

Additionally, collagen powders have the potential to satisfy many requirements of an ideal adjunctive wound therapy. Collagen is biodegradable, non-toxic, and naturally biocompatible with low antigenicity.[3] Collagen itself promotes thrombosis and hemostasis while having hydrophilic properties useful for absorbing fluid in exuding wounds and maintaining moisture balance.[12,13] Collagen is also a poor culture medium and can be left in wounds without risk of enhancing bacterial growth.[14] Lastly, collagen powders are easy to apply and are indicated for use in most wound types, including infected wounds.

We have designed a pilot study to investigate the effect and potential utility of topical Nuvagen™ (collagen powder) on the rate and quality of wound healing in healthy volunteers using the punch biopsy method. After inducing an acute full-thickness wound, the rate of complete healing of a wound treated with topical Nuvagen™ (collagen powder) will be compared to the rate of complete healing of a wound treated with primary closure with sutures, the current gold standard. Qualification and semi-quantification of histologic and immunohistochemical markers will be used to assess the maturity and structural stability of the wound bed. Positive findings would suggest that Nuvagen™ (collagen powder) may be capable of stimulating the healing of acute wounds in a similar or even superior manner to primary closure, suggesting collagen powder may be used in place of sutures, and encouraging further studies to characterize its therapeutic potential in dermatologic surgery.

3. RESEARCH DESIGN AND METHODS

3.1 Overview of Design

All participants will be patients that have been screened at the Department of Dermatology at the George Washington Medical Faculty Associates. Individuals taking part in the study will meet all of the inclusion criteria and none of the exclusion criteria (see Section 3.2). After completing the screening phase, subjects will receive 2 punch biopsy wounds on the thighs of each leg in their first study visit. Prior to dressing the wounds, 1 wound will be treated with primary closure, while the other will be treated with Nuvagen™ (collagen powder). Each wound will continue to be treated with the treatment designated in visit 1 (primary closure or collagen powder) for the duration of the 12-week study. After visit 1, patients will be provided with instructions and supply for daily self-administration of Nuvagen™ (collagen powder) for the wound designated for collagen powder treatment. Subjects will return weekly for 2 weeks, for suture removal if needed, photographs, wound measuring, assessment of healing, assessment of adverse events, and determination for need of further treatment with Nuvagen™ (collagen powder). Subjects will then return at week 4 for 2 additional punch biopsies at the same sites wounded at the first visit, and will be provided with similar treatment instructions as they were provided in week 1. Patients will return in 2 weeks (visit 5) for suture removal, photographs, wound measuring, assessment of healing, assessment of adverse events, and determination for need of further treatment with Nuvagen™ (collagen powder). The last study visit will be a 6 weeks after visit 5,

during which wounds will be measured and assessed for healing, while subjects will be photographed and assessed for adverse events.

Study Population

3.2 Sample Size, Inclusion and Exclusion criteria

We will enroll a total of 8 patients for this study. The inclusion and exclusion criteria for the study are as follows:

Inclusion criteria:

- Outpatient, male or female subjects of any race, 18-75 years of age
- Able to understand the requirements of the study and sign Informed Consent/HIPAA Authorization forms
- Patients willing to refrain from using topical medications to punch biopsy sites
- Patients who are willing to follow protocol instructions and return for follow-up visits
- Patients that are able to give signed informed consent for the study

Exclusion:

- Patients less than 18 years and greater than 75 years of age
- Patients that have any medical or skin condition that could impair wound healing
- Patients that have used systemic medications that suppress the immune system within 5 half-lives (if known), or 2 months of enrollment (i.e. corticosteroids)
- Patients that have applied topical steroids to the thigh(s) in the 2 weeks prior to enrollment
- Patients that are currently participating in an investigational study of a drug or device or have participated within 4 weeks of enrollment
- Patients that in the opinion of the investigator demonstrate evidence of unwillingness or inability to follow instructions or to complete the study
- Patients currently using systemic antimicrobials
- Patients with a history of diabetes mellitus
- Patients with a history of bleeding disorders or concomitant treatment with aspirin or anticoagulants (including heparin, low molecular weight heparin, warfarin, fondaparinux, or rivaroxaban)
- Patients with a history of keloids or hypertrophic scars
- Patients with other conditions considered by the investigator to be reasons for disqualification that may jeopardize subject safety or interfere with the objectives of the trial (e.g., acute illness or exacerbation of chronic illness)
- Patients with a known allergy or sensitivity to any component of the test medication (including bovine and/or collagen products) or local anesthetic agent used
- Current or previous users of tobacco products
- Recent alcohol or drug abuse is evident

- Pregnant females or nursing mothers. Eligible women of reproductive age will be required to have a negative urine pregnancy test at screening. They will also be required to be on at least 1 reliable form of effective birth control [examples: *barrier method (condoms, diaphragm), oral, injectable, implant birth control or abstinence*] during the course of this study and 30 days following the last treatment period.

3.3 Screening Phase

Prior to wounding and treatment, potential participants will be evaluated for study eligibility. All inclusion and exclusion criteria must be met prior to study entry and enrollment.

The patient's first visit will comprise both screening and wounding. During the screening phase, after obtaining signed informed consent, subject's personal data will be recorded. Data will include age, gender, medical and medication history and skin type. The physical examination will be performed and vital signs will be obtained. If the subject qualifies, visit 1 procedures will follow in the same visit.

3.4 Treatment Phase

On the first visit, eligibility criteria will be confirmed. Investigators will select 1 area on each anterior, proximal thigh of each volunteer where one 4 mm punch biopsies will be performed. The 2 areas will be cleansed using an alcohol swab, and anesthetized using 1 mL of 2% lidocaine with epinephrine. An Integra Miltex 4.0 mm Standard Biopsy Punch instrument will be used to create 2 full-thickness wounds, one on each thigh on each leg. Hemostasis will be obtained using Aluminum Chloride 20%, application of pressure, and gauze. Each wound will be photographed with a measurement instrument and then cleaned with normal saline prior to treatment. One wound will be selected for treatment with collagen, while the contralateral wound will undergo primary closure with sutures (1-2 epidermal sutures, 4-0 Prolene™ Polypropylene Sutures, Ethicon, Somerville, NJ).

The wound selected for collagen treatment will receive 1 gram of topical Nuvagen™ (collagen powder) directly on the wound with pressure applied using a dry gauze and forceps. Once hemostasis has occurred between 1 and 5 minutes later, the wound will be covered with a sterile dressing. The other wound will undergo primary closure. Both punch biopsy sites will be covered with a non-adherent dressing and a waterproof film dressing.

The patient will be provided with a 2-week supply of Nuvagen™ (collagen powder) in 1-gram containers and dressings along with instructions. Instructions will detail the following procedure: 1) Irrigate the wound with tap water or saline solution, 2) Dry the wound with a dry gauze pad pat using gentle pressure, 3) Apply 1 gram of Nuvagen™ (collagen powder) to the wound, and 4) Apply a sterile dressing which covers the entire wound. Assistance from a caregiver will be permitted in any instance in which this procedure is difficult for the patient to carry out for him or herself. This procedure is to be repeated daily for 2 weeks. On days of study visits, patients will be instructed to bring the day's 1 gram of Nuvagen™ (collagen powder) to the clinic for study coordinators to apply the corresponding day's collagen treatment.

One week after wounding, the patient will return for visit 2. Patients will perform the Patient Overall Assessment Score, wounds will be measured, photographs will be taken, and inquiry will be made about any possible adverse events. The patient's wound treated with collagen will then be treated and dressed in the office.

One week after visit 2, the patient will return for visit 3. Sutures will be removed, and the wound treated with collagen will be evaluated for further collagen treatment. The principal investigator will determine if another 2 weeks of Nuvagen™ (collagen powder) treatment will be warranted. If further collagen treatment is advised, the patient will be provided with another 2-week supply of Nuvagen™ (collagen powder) and dressings. Patients will perform the Patient Overall Assessment Score, wounds will be measured, photographs will be taken, and inquiry will be made about any possible adverse events. The wound treated with collagen will be treated and dressed in the office.

Two weeks after visit 3, the patient will return for visit 4. Both wound sites will be photographed and then biopsied again following the procedure from visit 1. Each wound will be treated identically (either with primary closure or with collagen powder) as it was in visit one. Patients will be provided with adequate supplies for at-home collagen treatment for 2 weeks. Patients will perform the Patient Overall Assessment Score, wounds will be measured, photographs will be taken, and inquiry will be made about any possible adverse events.

Two weeks after visit 4, the patient will return for visit 5. Sutures will be removed, and the wound treated with Nuvagen™ (collagen powder) will be evaluated for further collagen treatment. The principal investigator will determine if another 2 weeks of Nuvagen™ (collagen powder) treatment will be warranted. If further collagen treatment is advised, the patient will be provided with another 2-week supply of Nuvagen™ (collagen powder) and dressings. Patients will perform the Patient Overall Assessment Score, wounds will be measured, photographs will be taken, and inquiry will be made about any possible adverse events. The wound treated with collagen will be treated and dressed in the office.

Six weeks after visit 5, the patient will return for visit 6. Patients will perform the Patient Overall Assessment Score, wounds will be measured, photographs will be taken, and inquiry will be made about any possible adverse events.

3.5 Study Completion and Withdrawal Criteria

It is the right and duty of the Investigator to discontinue the study participation of a subject when the subject's health or well-being is threatened by continuation in the study. Such subjects should be withdrawn from the study and not continued under a modified regimen.

The following are circumstances that would result in the subject's discontinuation from the study:

- The subject experiences a serious adverse event rendering them unable to continue study participation;
- The subject is unable to physically or mentally tolerate the use of the lest medication;
- An exclusion criterion becomes apparent at any time during the study, or the subject voluntarily withdraws.

In the event of premature discontinuation from the study, the Investigator should determine the primary reason for discontinuation. A subject who is withdrawn from the study prior to initiation of treatment may be replaced.

3.6 Strength and Limitations of Proposed Research

Strengths of the study include its relatively simple design and the potential for its completion within a short period of time due to the small number of participants (eight).

Limitations of this study include the inability to conduct robust statistical analysis due to its small sample size. However, the results of this project may lead to the development of larger studies that will have the statistical power to detect improvements.

4. DATA VARIABLES, COLLECTION, AND EVALUATION

4.1 Data Variables

The following data will be evaluated in patients fulfilling inclusion and exclusion criteria and who agree to participate in the study.

1. ***Sociodemographic Variables***: Age, gender, and race.

2. ***Clinical History***:
   a. **Past medical history**: All current and past medical conditions will be documented, allowing us to screen for the presence of any condition that, in the opinion of any of the investigators, would make the patient unsuitable for study inclusion. Allergies to medications will also be noted.
   b. **Current medications**: All medications will be recorded.
   c. **Social History**: We will screen for the presence of tobacco product use in addition to any drug or alcohol abuse within the past 6 months as determined by the medical record or patient interview.

4.2 Wound specific variables

Characterization of wound healing: Photographs of the wounds will be taken on week 0, 1, 2, and 4, at which time the areas will be re-sampled for histologic analysis, and then photographed again at the 4 week time point, followed by week 6 and then 12. Photographs will be used to follow gross visual wound healing as assessed by the area of the wound uncovered by the migrating epithelia as measured digitally using ImageJ software. Digital measurements will be performed by 2 different members of the research team.

Morphometric analysis of wound sections*:* Wound re-epithelialization will be measured in Hematoxylin and Eosin stained sections from the center of the wound. Dermatopathology procedures will be carried out by HistoWiz, Inc., Broolyn, NY. The distance between the wound edges, defined by the distance between the first hair follicle encountered at each end of the wound, and the distance that the epithelium had traversed into the wound, will be analyzed using

ImageJ. The percentage of re-epithelialization [(distance traversed by epithelium)/(distance between wound edges) x 100] will be calculated and averaged for 2 sections per wound. The quality of the epidermis and dermis will also be evaluated as a measure of wound maturation at the 4 week time point.

Collagen deposition*:* Dermatopathology procedures will be carried out by HistoWiz, Inc., Broolyn, NY. Staining will be performed using Masson's trichrome stain and the percentage of blue collagen-stained area relative to the total area of the wound bed after taking digital images. This will be quantified by counting the number of pixels staining above a threshold intensity and normalizing to the total number of pixels.

Angiogenesis*:* Dermatopathology procedures will be carried out by HistoWiz, Inc., Broolyn, NY. Wound sections will be stained using CD31 antibody (also called platelet-derived endothelial cell adhesion molecule-1). Digital images at 40x magnification covering the majority of the wound bed will be taken. The percent area stained in each image will be quantified by counting the number of pixels staining above a threshold intensity and normalizing to the total number of pixels. Threshold intensity will be set such that only clearly stained pixels are counted. Staining identified as artifact, large vessels, and areas deemed to be outside the wound bed will be excluded.

Patient Overall Assessment Scale. Patients will assess the overall improvement of their wounds using a 4-point scale, where 1 = excellent improvement, 2 = good / moderate improvement, 3 = no change, 4 = worsening. This assessment will be performed prior to every follow up visit after wounding at week 0, and upon study completion for each wound. Improvement will be indicated by a decrease by ≥ 1 point compared to the previous assessment. Worsening will be indicated by an increase in this scale by ≥ 1 point compared to the previous assessment. Patients will also be asked preference between the Nuvagen^{TM} (collagen powder) and primary closure.

Pruritus Numerical Rating (NRS) Scale. Patients will also score pruritus on the validated NRS scale, which ranges from 1-10. This assessment will be performed prior to every follow up visit after wounding at week 0, and upon study completion for each wound. Improvement will be indicated by a decrease by ≥ 2 points compared to the previous assessment. Worsening will be indicated by an increase in this scale by ≥ 2 points compared to the previous assessment.

4.2 Data Collection

All data variables will be stored in a secure database using coded identifiers in place of name. Most of the data will initially be recorded on approved study forms containing the participant's study ID code. After transfer of data into the computerized database, all paper forms will be kept in a secure locked cabinet within a locked office within the department. Photographs will be stored with a secure electronic file and will be labeled with the participant's study ID code and date they were taken.

4.3 Statistical Considerations

Since this is a pilot study, we will perform only basic descriptive statistical analysis of our results. Due to the small number of subjects, it is unlikely that any statistical test would have the power to detect a significant difference in any of the measured variables. Instead, we will look at

wound closure, quality of forming scar histologically, and assess patient experience and preference. The results of this project may lead to the development of larger studies that will have the statistical power to detect significant differences in these variables after treatment with Nuvagen™ (collagen powder).

5. HUMAN SUBJECTS PROTECTION

5.1 Subject Selection

At the time of recruitment, each potential subject will be provided with an informed consent form to read. The potential subject will be allowed to take the consent form home to discuss its content with family members or friends. All individuals will be given a contact number that they may call to obtain answers to any questions regarding the study and their participation. All subjects must sign a consent form prior to any study procedures being performed.

Involvement of special subjects such as pregnant women, institutionalized individuals, prisoners, or impaired and non-competent individuals are not anticipated in this study. Eligible women of reproductive age will be required to be on 1 reliable form of effective birth control during the treatment period. They will also be required to have a negative urine pregnancy test during the screening period and prior to each treatment session for the duration of the study. Individuals from all racial/ethnic groups, regardless of gender, and those that are economically and educationally disadvantaged are eligible for this study if they meet the eligibility requirements.

5.2 Risks and Benefits

Risks from punch biopsies include the possibility of pain, bleeding, itching, or infection at the biopsy site. Further, there is the possibility of persistent bruising, swelling, or scarring. Pain at the biopsy site will be minimized by use of a local anesthetizing agent. Excessive bleeding will be prevented by excluding patients with known coagulation disorders, patients taking aspirin, or patients taking anticoagulants (including heparin, low molecular weight heparin, warfarin, fondaparinux, or rivaroxaban). Further, bleeding will be minimized by applying adequate pressure using gauze on the biopsy sites. Previous work has shown that wound infections can occur in about 1 in every 6 outpatient skin biopsies.[15] This study failed to subcategorize infection rate for each biopsy type, however, and included patients with medical co-morbidities which contribute to poor wound healing and development of infections. Risk of infection in our study will be minimized by excluding smokers, patients with diabetes mellitus, and patients with other medical or skin conditions that impair wound healing. Collagen is also a poor culture medium and can be left in wounds without risk of enhancing bacterial growth.[14]

Patients may have an allergy or allergic reaction to the numbing agent used, but every attempt to prevent this will be made by asking the patient about his or her allergy history. Furthermore, there is a potential risk of allergy to the interventional type I Nuvagen™ (collagen powder). Although studies have shown that it is possible to develop monoclonal antibodies to mammalian collagen, type I collagen has shown to be very poorly immunogenic.[3,16,17] Nonetheless, there have been sparse reports of allergy to topical bovine-derived collagen.[18] Minor amounts of non-collagen proteins in collagen products have also been implicated in immunogenicity of collagen

products.[19] Risk of allergy to the investigational agent used will be minimized further by excluding patients with history of known allergy or sensitivity to any component of the test medication (including bovine and/or collagen products).

There is a potential risk of loss of privacy. All possible precautions will be taken to respect privacy and the confidentiality of patient information, and a secured database with coded identifiers will be used to keep privacy breach risk minimal.

There is no guarantee of direct benefit from study involvement. Information from this study will be used to improve wound care practices after skin biopsies. Subjects could receive up to $590.00 total for participation if all study visits are attended. For each biopsy procedure, subjects will be compensated $125.00, for a total of $500.00 for all 4 planned biopsies obtained during the study (2 biopsies in visit 1, and 2 more biopsies in visit 4). In addition, subjects will be reimbursed $15.00 for travel expenses for each visit, for a total of $90.00 for all 6 planned study visits. Subjects will only receive payment for study visits attended and biopsy procedures already performed should he or she decide to stop the study early. Payments are to be made in full to the subject within 2 months after the final visit for the study.

5.3 Confidentiality

All information collected as part of this study will be treated as confidential. Subject consent forms and data collection sheets will be stored in a locked office in the MFA Dermatology Department. These documents will be accessible to study investigators only. A computer database will be created. This database will not contain any subject identifiers. The data within the database will be labeled with each subject's study identification number. Database information will be maintained in a password-protected file. All the data included in this study will only be accessible to the investigators listed on the title page, and they will not be released for use by other researchers.

5.4 Adverse Events and Safety Monitoring

Patients will be closely monitored for any adverse events experienced during this study, as described in Section 5.2: Risks and Benefits. Adverse events monitoring will be performed by the principal investigator during each treatment session starting at visit 2. Any event occurring more than 8 weeks after the second pair of punch biopsies at visit 4 will not be considered an adverse event. Patients will be educated as to the possible side effects and complications related to punch biopsies and Nuvagen™ (collagen powder), and will be instructed to notify any investigator should they experience signs or symptoms of an adverse event. Because the anticipated risks associated with this study are low, we feel that an independent monitoring board will not be necessary.

All events that meet the Prompt Reporting Requirements (HRP-801) (including but not limited to new or increased risk, harm experienced by the subject, noncompliance with the federal regulations governing human research, and state medical board actions) will be reported to the GW OHR via a Problem Report and Promptly Reportable Information Form (HRP-204) within 5 business days of knowledge of the event.

Table 1: Example Study Schedule of Events*

Week	Screen**	W0**	W1	W2	W4	W6	W12
Visit	**V1**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**
Informed Consent	X						
Clinical History/ Screening Form	X						
Wounding (punch biopsies)		X			X		
Wound measurements		X	X	X	X	X	X
Patient Overall Assessment Scale			X	X	X	X	X
Pruritus Numerical Rating Scale			X	X	X	X	X
Photographs		X***	X	X	X****	X	X
Adverse Events			X	X	X	X	X

*Actual schedule and time between treatments may vary for each subject. **Screen and W0 procedures will take place in the same visit, V1. ***Photographs will be obtained immediately after the biopsy procedures. ****Photographs will be obtained immediately before and immediately after the biopsy procedures.

6. REFERENCES

1. Attinger CE, Janis JE, Steinberg J, Schwartz J, Al-Attar A, Couch K. Clinical approach to wounds: débridement and wound bed preparation including the use of dressings and wound-healing adjuvants. *Plast Reconstr Surg*. 2006;117(7 Suppl):72S-109S. doi:10.1097/01.prs.0000225470.42514.8f.
2. Fletcher J. Differences between acute and chronic wounds and the role of wound bed preparation. *Nurs Stand*. 2008;22(24):62-68. doi:10.7748/ns2008.02.22.24.62.c6412.
3. Chattopadhyay S, Raines RT. Review collagen-based biomaterials for wound healing. *Biopolymers*. 2014;101(8):821-833. doi:10.1002/bip.22486.
4. Purna SK, Babu M. Collagen based dressings - A review. *Burns*. 2000;26(1):54-62. doi:10.1016/S0305-4179(99)00103-5.
5. Fleck CA, Simman R. Modern collagen wound dressings: Function and purpose. *J Am Col Certif Wound Spec*. 2010;2(3):50-54. doi:10.1016/j.jcws.2010.12.003.
6. GOULD BS, WOESSNER JF. Biosynthesis of collagen; the influence of ascorbic acid on the proline, hydroxyproline, glycine, and collagen content of regenerating guinea pig skin.

J Biol Chem. 1957;226(1):289-300.
7. Brett D. A Review of Collagen and Collagen-based Wound Dressings. *Wounds*. 2008;20(12):1-11.
8. Janis JE, Harrison B. Wound healing: part I. Basic science. *Plast Reconstr Surg*. 2014;133(2):199e-207e. doi:10.1097/01.prs.0000437224.02985.f9.
9. Stötzel S, Schurink M, Wienk H, et al. Molecular organization of various collagen fragments as revealed by atomic force microscopy and diffusion-ordered NMR spectroscopy. *ChemPhysChem*. 2012;13(13):3117-3125. doi:10.1002/cphc.201200284.
10. Landsman A, Taft D, Riemer K. The Role of Collagen Bioscaffolds, Foamed Collagen, and Living Skin Equivalents in Wound Healing. *Clin Podiatr Med Surg*. 2009;26(4):525-533. doi:10.1016/j.cpm.2009.08.012.
11. Ramadass SK, Perumal S, Gopinath A, Nisal A, Subramanian S, Madhan B. Sol-gel assisted fabrication of collagen hydrolysate composite scaffold: A novel therapeutic alternative to the traditional collagen scaffold. *ACS Appl Mater Interfaces*. 2014;6(17):15015-15025. doi:10.1021/am502948g.
12. Smith KJ, Skelton HG, Barrett TL, Welch M, Beard J. Histologic and immunohistochemical features in biopsy sites in which bovine collagen matrix was used for hemostasis. *J Am Acad Dermatol*. 1996;34(3):434-438. doi:10.1016/S0190-9622(96)90435-1.
13. Boateng JS, Matthews KH, Stevens HNE, Eccleston GM. Wound healing dressings and drug delivery systems: A review. *J Pharm Sci*. 2008;97(8):2892-2923. doi:10.1002/jps.21210.
14. Motta G, Ratto GB, Barbieri A De, et al. Can heterologous collagen enhance the granulation tissue growth? An experimental study. *Ital J Surg Sci*. 1983;13(2):101-108.
15. Wahie S, Lawrence CM. Wound complications following diagnostic skin biopsies in dermatology inpatients. *Arch Dermatol*. 2007;143(10):1267-1271. doi:10.1001/archderm.143.10.1267.
16. Ramshaw JAM, Werkmeister JA, Glattauer V. Collagen-based biomaterials. *Biotechnol Genet Eng Rev*. 1996;13(1):335-382. doi:10.1080/02648725.1996.10647934.
17. Werkmeister JA, Ramshaw JA, Ellender G. Characterisation of a monoclonal antibody against native human type I collagen. *Eur J Biochem*. 1990;187(2):439-443. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2105219.
18. Mullins RJ, Richards C, Walker T. Allergic reactions to oral, surgical and topical bovine collagen. Anaphylactic risk for surgeons. *Aust N Z J Ophthalmol*. 1996;24(3):257-260. doi:10.1111/j.1442-9071.1996.tb01589.x.
19. DeLustro F, Smith ST, Sundsmo J, Salem G, Kincaid S, Ellingsworth L. Reaction to injectable collagen: results in animal models and clinical use. *Plast Reconstr Surg*. 1987;79(4):581-594.