**NeXtGen Biologics, Inc.**
**12085 Research Drive, Box 17**
**Alachua, FL 32615**

**Jonelle Toothman**
**800-209-7190 ext 2**
**support@nextgenbiologics.com**

CONFIDENTIAL COMMUNICATION

**Skin Prick Test (SPT): 20 Patients**

ABBEVIATIONS

| | | |
|---|---|---|
| AE | – | Adverse Event |
| CFR | - | Code of Federal Regulations |
| CRF | - | Case Report Form |
| GCP | - | Good Clinical Practice |
| ESS | - | Erythema Scoring Scale |
| HIPAA | - | Health Insurance Portability and Accountability Act |
| ICF | - | Informed Consent Form |
| IRB | - | Institutional Review Board |
| PI | - | Principal Investigator or designee |
| RIPT | - | Repeated Insult Patch Test |
| SAE | - | Serious Adverse Event |
| SOP | - | Standard Operating Procedure |
| SPT | - | Skin Prick Test |

DEFINITIONS

**Adverse Event (AE):** Any negative experience that a volunteer has during the course of a clinical trial, including new or worsening symptoms or laboratory abnormalities.

**Belmont Report:** A document entitled "Ethical Principles and Guidelines for the Protection of Human Subjects of Research" that was produced by the National Commission for the Protection of Human Subjects of Biomedical and Behavior Research in 1978. The Belmont Report identifies three fundamental ethical principles for all human subject research: respect for persons, beneficence, and justice.

**Blinding:** When research subjects are unaware of the assigned "treatment." In a single-blinded trial, the subjects do not know what treatment they are receiving. In a double-blinded trial, the subjects and the investigators are unaware of the treatment assigned, as are the monitors and statisticians in some cases.

**Case Report Form (CRF):** A record of information collected on each subject during the clinical trial.

**Clinical Investigation:** A systematic trial designed to evaluate a test material in humans.

**Clinical Research:** A trial in human subjects.

**Clinical Trial:** Any investigation in humans meant to determine properties of a test material.

**Ethics Committee:** An independent group of medical and nonmedical people who verify the integrity of a trial and ensure the safety, integrity, and human rights of the subjects.

**Exclusion Criteria:** Characteristic that would prevent a subject from being eligible to participate in a research trial, as specified in the protocol.

**Good Clinical Practices (GCP):** International ethical and scientific quality standards for the design, conduct, monitoring, recording, auditing, analysis, and reporting of trials.

**Health Insurance Portability and Accountability Act (HIPAA):** Legislation passed in 1996 that includes a privacy rule creating national standards to protect personal health information.

**Inclusion Criteria:** A list of requirements that a subject must meet to be eligible to participate in a research trial, as specified in the protocol.

## 1 PROTOCOL SUMMARY

| | |
|---|---|
| Title: | Skin prick testing of healthy adult volunteers to investigate the potential of allergy to NeoMatriX™ Wound Matrix |
| Design: | Unblinded |
| Test Articles: | 1. NeoMatriX™ Wound Matrix, an investigational device [redacted] |
| Duration: | 2-3 days |
| Subjects: | Sufficient healthy volunteer subjects so that 20 will complete the study |
| Observations: | Scoring of any skin reactions at 15 minutes, 6 hours and 1-2 days after prick test |
| Location: | Clinical Research Center, UB Clinical and Translational Research Center<br>875 Ellicott Street, Buffalo, NY |

## 2 KEY STUDY PERSONNEL AND RESPONSIBILITES

| Key Role | Key Personnel | General Responsibilities |
|---|---|---|
| Principal Investigator (PI) | Stanley Schwartz, MD, PhD<br>Chief, Division of Allergy, Immunology & Rheumatology<br>SUNY at Buffalo<br>Buffalo General Medical Center | The PI is responsible for ensuring sufficient resources are available to conduct the study, for investigating and reporting any serious adverse events to the Sponsor, the study design, compiling the results and writing the clinical report. |

| | | |
|---|---|---|
| Study Coordinator | Robin Stein, RN, BSN | The Study Coordinator will be responsible for conducting the study on a daily basis. |
| Monitor | NeXtGen Appointee | |
| Project Coordinator | Jonelle Toothman | Sponsor representative who is the primary point of contact and represents the Sponsor (NeXtGen Biologics) |

## 3 STUDY VISIT CHART

| Day | 01 | | 02-03 |
|---|---|---|---|
| Prick Test Performed | ✓ | | |
| Clinically Evaluate Prick Site | 15 mins<br>✓ | 6 hours<br>✓ | <br>✓ |

## 4 INTRODUCTION, BACKGROUND AND OBJECTIVE

Skin prick tests for immediate hypersensitivity have been the gold standard as assays for allergies since the 1860s and are currently used by allergists identify allergic reactions to specific allergens. These tests are minimally invasive and are used on patients of all ages from infants to seniors. They have the advantage of being highly sensitive and specific and produce rapid results.

Therapies for facilitating wound healing currently are limited especially with regard to chronic wounds and remain a significant unmet need. Chronic wounds including non-healing skin ulcers such as pressure ulcers, diabetic neuropathic ulcers and vascular insufficiency ulcers, are a source of substantial morbidity and mortality for hospitalized patients and residents of nursing homes. Non-healing and chronic wounds increase the risk of infection and can result in limb amputation and death.

Numerous topical treatments for non-healing wounds have produced mixed results and an ideal treatment remains to be achieved. The test product of this proposal, NeoMatriX™ Wound Matrix, is a wound dressing for the management of wounds, acute and chronic. NeoMatriX™ Wound Matrix has the features of a biocompatible biomaterial. Despite its stringent preparation protocols and having completed a range of biocompatibility testing, prior to utilization in human clinical trials, it remains to be tested in a small pilot study to determine if it can elicit any allergic responses. While it is highly unlikely that any study subject will have de novo, immediate allergic reactions to NeoMatriX™ Wound Matrix, the current protocol is designed to document this and determine whether study subjects may show any delayed allergic reactions.

The objective of this study is to investigate the potential of NeoMatriX™ Wound Matrix to cause an allergic response in healthy volunteers. The biomaterial is applied to the forearm of study subjects, a drop of saline solution is placed on the membrane and using a standard skin prick test device the underlying epidermis is superficially pricked through the overlying membrane. After the test, the area of the skin is observed after 15 minutes and 6 hours to see if a reaction develops. The test site will again be evaluated at 1-2 days after the initial prick test. The skin will be examined for a "wheal"—a raised, itchy bump and surrounding erythematous "flare".

[redacted] and flare whose diameter can be measured and exceed a wheal at the negative control by 4 mm. Pricking through a drop of saline serves as a negative control that is not expected to produce a significant wheal and flare. [redacted], to reiterate, this is completely unanticipated but remains to be proven.

## 5 STUDY DESIGN

The prick test is not blinded.

## 6 SELECTION OF SUBJECTS

### 6.1 Screenings

A sufficient number of subjects will be recruited to ensure a minimum of 20 subjects complete the study. Subjects must satisfy the inclusion and exclusion criteria, must be prepared to accept the prohibitions and restrictions and must give written informed consent.

The suitability of each potential subject will be confirmed before their acceptance by review of a study specific pre-treatment questionnaire.

### 6.2 Inclusion criteria

6.2.1 Healthy volunteers, of either sex, aged at least 18 years.

6.2.2 Completed written informed consent and receive a copy of their executed ICF.

6.2.3 Volunteers must be capable of understanding and following directions in English.

### 6.3 Exclusion criteria

6.3.1 Pregnancy or lactation.

6.3.2 Inadequate or non-existent contraception (women of child bearing potential only).

6.3.3 A current skin disease of any type apart from mild facial acne (e.g. eczema, psoriasis).

6.3.4 Heavy alcohol consumption.

6.3.5 Current use or history of repeated use of recreational drugs.

6.3.6 A febrile illness lasting more than 24 hours in the six days prior to test.

6.3.7 Significant past medical history of hepatic, renal, cardiac, pulmonary, digestive, hematological, neurological, locomotor or psychiatric disease.

6.3.8 [redacted]

[redacted]

6.3.10 [redacted]

[redacted] erials.

6.3.12 Current treatment by a physician for allergy unless physician consulted by Investigator and participation approved.

6.3.13 Participation in a repeat insult patch test (RIPT), skin prick test (SPT) or follow-up work within the last month.

6.3.14 Sensitization or questionable sensitization in a RIPT or SPT.

6.3.15 Recent immunization (less than 10 days prior to prick test).

6.3.16 A medical history indicating atopy or severe dematographism.

**6.4 Prohibitions and restrictions for the duration of the study**

6.4.1 No use of aspirin or non-steroidal anti-inflammatory or corticosteroid drugs for the duration of the study.

6.4.2 [redacted]

6.4.3 [redacted]

## [redacted] METHOD

**7.1 Test Articles – NeoMatriX™, extracellular matrix xenograft, 8mm disc**

**7.2 Negative Skin Test Control– sterile normal saline**

**7.3 Positive Skin Test Control – Sterile histamine** [redacted]

**7.4 Materials**

- Test article NEOMATRIX™ 8mm disc
- Positive control solution
- Negative control solution
- [redacted]
- Sharps container for disposal of picks
- Marker pen for the skin
- Ruler for measuring reactions
- Sterile cotton balls for wiping skin
- Gloves (latex-free)
- Recording sheets, Case Report Forms
- Syringes and needles
- Cetirizine 10 mg tablets
- Hydrocortisone cream 1%

### 7.5 Devices used for skin prick testing

- [redacted]

### 7.6 Procedure

The subject needs is placed in a comfortable position with volar surface of the forearm [redacted] The room should be private and at a comfortable temperature. It is advisable to provide the patient with a magazine or something to occupy themselves for the 15-20 minutes that is required for the test (and to distract them from any discomfort).

The test area should be clean, free from moisturizers and area for skin pricks should be more than 5 cm from the wrist and 3 cm from the antecubital fossa and marked by numbers on the skin to identify the test article, negative control and positive control. Pricks should be made immediately adjacent to the numbers to avoid confusion between allergens. Prick tests should be at least 2 cm apart to avoid overlapping reactions and false-positive results.

[redacted]

Positive and negative controls will be applied directly with the Picks.

A separate Pick is used to prick each site. For example, there should be 3 Picks used per subject, one for each test site. Prick each site separately.

[redacted]

[redacted]

### 7.7 Grading of Test Sites - Reading Results

- Initial reading after 15 minutes looking for the presence or absence of a wheal and flare at all three sites and measuring the diameter of both the wheal and flare if present. The wheal at the positive control must exceed a wheal at the negative control by 4 mm.
- A second reading is conducted about 6 hours after the SPT administration and similar measurements are taken.
- The subjects return to the office 1-2 days after the initial administration for a third test reading and measurements.
- A positive reaction is present when there is a measurable wheal of >3 mm . Small wheals are confirmed by palpation. A flare alone is measured but is not of any clinical significance.

### 7.8 Characteristics of Wheal and Flare

Wheals are determined by palpation or oblique lighting and the diameter is measured in mm. If the wheal is not asymmetrical two diameters are measured (smallest and largest) and an average value is calculated. Flares, areas of erythema, are similarly measured but are not considered clinically significant.

## 8 SAFETY - ADVERSE EVENTS

### 8.1 Relationship to Test Material

The relationship or association of the AE to the test material will be characterized as unlikely, possible or probably. Assessments shall be recorded on the CRF.

| Causality Term | Assessment Criteria |
|---|---|
| Probable | Event or laboratory test abnormality, with plausible time relationship to test material exposure |
| | Unlikely to be attributed to condition (or disease) or other products in use by subject |
| | Response to withdrawal clinically reasonable |
| | Rechallenge satisfactory or not required |
| Possible | Event or laboratory test abnormality, with reasonable time relationship to test material intake |
| | Could also be explained by condition (or disease) or other products in use by subject |
| | |
| Unlikely | Event or laboratory test abnormality, with a time to test material intake that makes a relationship improbable (but not impossible) |
| | Condition (or disease) or other products provide plausible explanations |

For safety analyses, AEs that are classified as a possible or probable association to a test material shall be considered test material-related AEs.

Follow-up of the AE, after the date of discontinuation of exposure to test material is required if the AE or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the PI and to the Medical Monitor.

### 8.2 Severity of Adverse Events

The severity of each AE will be graded using the following criteria:

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| Grade | Description |
|---|---|
| 1 | Mild – Minor AEs requiring no specific medical intervention including asymptomatic laboratory findings only, or finding of marginal clinical relevance |
| 2 | Moderate – AEs including urticaria requiring minimal, local, and/or noninvasive intervention |
| 3 | Severe – Severe and undesirable AEs involving significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; or therapeutic endoscopy or operation |

| | |
|---|---|
| 4 | Life-threatening or debilitating – AEs complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, or sepsis. Also, life-threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation. |
| 5 | Death |

If the Grade changes within a day, the maximum Grade shall be recorded.

If the Grade changes over a longer period of time, the changes shall be recorded as separate events (having separate onset and stop dates for each grade).

### 8.3 Serious Adverse Events

An SAE is defined as any AE that suggests a significant clinical hazard, contraindication, side effect, or precaution. This includes any event which:

- Results in death
- Is life-threatening
- Results in persistent or significant disability/incapacity
- Results in or prolongs an existing inpatient hospitalization
- Results in a congenital anomaly/birth defect
- Is an important medical event based on medical judgment, which jeopardizes the subject and requires medical or surgical intervention to prevent one of the outcomes listed above.

The PI will evaluate all serious AEs as to their intensity, relation to test material, outcome, and action taken.

Management of Adverse Events

No adverse reaction is anticipated. The patient will be evaluated at 15 minutes after SPT and then again at 6 hours after SPT. Minor adverse events (e.g. itching at the skin test site) will be examined by the nursing staff of the CRC; the PI will be contacted as needed.

- Over-the-counter (OTC) antihistamines will be given, which relieve minor symptoms. Antihistamines prevent symptoms such as hives by blocking histamine receptors.
- Persistent itching, swelling or redness, will be reduced with ice and at the test site we shall apply 1% hydrocortisone cream one time.
- Should the subject develop urticaria they will be provided with a 5 day course of antihistamine (10 mg cetirizine orally once daily)

While serious adverse events are not anticipated the most likely serious event would be a systemic allergic reaction or anaphylaxis which would most likely occur within 20 minutes while the subject is under close evaluation at the CRC.

- The subject will be assisted to lie on their back with feet raised about 12 inches, clothing loosened to ease breathing, covered with a blanket and coached to stay calm. They will be turned on their side if vomiting or bleeding.
- Epinephrine (adrenaline) may be used, depending on the severity.

- Should anaphylaxis occur Epinephrine 1:1000 will be administered immediately by intramuscular injection and repeated every 10 min. as needed. The PI will be summoned stat and the subject will be transported to the Emergency Department of the Buffalo General Medical Center located on the ground floor of the same building housing the Clinical Research Center.

## 9 REPORTING

9.1 Evaluation of Results – this simple study design, consists of only one physiological measure (that of wheal/flare) measured at 3 time points: T1-15 minutes after SPT, T2=6hr after SPT and T3=24-48 hrs. after SPT. Our power analysis calculation indicates at a confidence level of 99% and confidence interval of 1.35, we will need a sample size of n=20. No statistical analysis will be performed. No significant effect or interaction effects are anticipated hence the small sample size.

9.2 Interim Reports – unexpected findings will be reported to the Sponsor. At the conclusion of the study, a draft report will be sent to the Sponsor and contain all of the information to be included on the final report. Comments made by the Sponsor may be incorporated into the draft report after which it will be issued as the final report and signed by the PI.

9.3 Correction or Additions to the Final Report – the sponsor will review the draft final report and make necessary edits prior to the final report being issued.

TEST ARTICLE PRECAUTIONS:

[redacted]

TEST ARTICLE POTENTIAL COMPLICATIONS:

[redacted]

TEST ARTICLE STORAGE:

[redacted]