A Randomized Study of Biologic Scaffolds and Esophageal Healing Following Endoscopic Resection

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A RANDOMIZED STUDY OF BIOLOGIC SCAFFOLDS AND ESOPHAGEAL HEALING FOLLOWING ENDOSCOPIC RESECTION

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IDE	Investigational Device Exemption
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
UADE	Unanticipated Adverse Device Effect
ECM	Extracellular matrix
EMR	Endoscopic mucosal resection
EGD	Esophagogastroduodenoscopy
BE	Barrett's esophagus

Study Summary

Title	A Randomized Study of the Use of Biologic Scaffolds in Constructive Healing Following Endoscopic Resection
Running Title	Biologic Scaffold Study
IRB Protocol Number	16-006909
Phase	Device Feasibility trial
Methodology	Randomized study
Overall Study Duration	1 year
Subject Participation Duration	12-16 weeks
Objectives	This study is being done to assess the efficacy of ECM products in promoting constructive healing as determined by the ability of the resection site to lift during follow-up.
Number of Subjects	20
Diagnosis and Main Inclusion Criteria	18 and older Male or female Able to provide consent Impending endoscopy that may require initial EMR evaluation of esophageal lesions
Study Device	The Acell MatriStem Surgical Matrix will cover the defect site following mucosal resection using endoscopy clips or X-tac
Duration of Exposure	The device will remain attached to the esophagus and integrate with native tissue over time
Reference therapy	There is no reference treatment for comparison
Statistical Methodology	Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the procedures described in this protocol, applicable United States government regulations and Mayo Clinic policies and procedures.

1.1 Background

The relatively recent introduction of endoscopic resection techniques (endoscopic mucosal resection [EMR] and endoscopic submucosal dissection [ESD]) have enabled endoscopists to excise larger amounts of tissue for accurate staging, and often curative treatment of early esophageal adenocarcinoma. These techniques have largely supplanted esophagectomy for small esophageal cancers which carries significant risks of morbidity and mortality.

One limitation of endoscopic resection, however, is the risk of esophageal stricture formation at the site of resection as a result of the inflammatory healing process that ensues after deep mucosal injury. The esophagus has limited regenerative ability and the default response is volume replacement of the defect with fibrosis.[1] Esophageal stricture can significantly impact a patient's quality of life and often requires treatment via multiple endoscopies for sequential pneumatic dilations, which carry risk of perforation. It has been reported that the risk of stricture formation is directly proportional to the size of the resection defect. Patients with mucosal defects greater than 75% of the esophageal circumference carry a significantly greater risk of stricture formation[2], and in patients with full circumferential resection the risk may be as high as 88%.[3, 4] Hence, extensive endoscopic resection typically give endoscopists pause. However, this risk of stricture is often justified by the need to excise the lesions en bloc to ensure accurate determination of depth of invasion and also to ensure the complete removal of the lesion.

Various interventions have been described in an effort to prevent stricture formation, including intralesional and systemic steroids, preventative balloon dilation, and prophylactic covered stent, but results are conflicting.[5] Currently, most endoscopists limit EMR to anticipated defects of 2 cm in diameter or 50% esophageal circumference in an effort to prevent stricture formation.

1.2 Investigational Device

Extracellular matrix (ECM) products such as MatriStem® Surgical Matrix produced by Acell (Columbia, MD)are approved by the US Food and Drug Administration (FDA) to aid in the healing of wounds like those created by EMR. ECM are composed of collagen and natural proteins that provide a re-absorbable scaffold for tissue remodeling have been investigated in preclinical as well as clinical pilot studies and found to facilitate constructive healing following EMR, meaning formation of site-specific functional tissue. Meanwhile, the inflammatory pathway that leads to stricture formation is diverted.

1.3 Preclinical Data

ECM are the result of minimally processing (i.e., decellularizing) biological tissue (e.g., porcine bladder), during which antigenic cellular components are removed while important proteins (e.g, glycosaminoglycans) and an ultrastructure consisting of collagen, fibronectin and basement membrane are preserved.[1, 6] These components retain their bioactivity in the host, and provide an environment that promotes cell adhesion and proliferation.[7] ECM scaffold degradation begins to occur rapidly after placement,[8, 9] reducing the potential for a chronic inflammatory process or foreign body reaction (as described with synthetic biomaterials[10]), and releasing cryptic peptides, including various cytokines and growth factors such as vascular endothelial growth factor and basic fibroblast growth.[11] These peptides have been shown to exert chemotactic and mitogenic effects on stem and progenitor cells *in vitro* that contribute to the constructive remodeling process.[12-14] ECM biologic scaffolds made of porcine-derived small intestinal submucosa have been successfully implanted without a keratonocyte layer in animal models. They were effective in reducing esophageal stricture after EMR, while facilitating constructive nonstenotic healing response with formation of all layers of the esophageal wall in a preclinical dog model.[15]

1.4 Clinical Data to Date

Observations on the use of ECM in the human esophagus have been reported in several series. Scaffolds from porcine urinary bladder were used to treat five BE patients with high-grade dysplasia/ esophageal adenocarcinoma and long-segment BE (8 to 13cm) following ESD.[8] The scaffold was applied using a temporary stent that was removed within 9-17 days, allowing scaffold integration with the underlying muscular wall of the esophagus. Complete epithelialization and formation of a new submucosal layer was appreciated during follow-up. All patients required transient postoperative dilation for mild stricture formation in areas not covered by ECM, but were able to then tolerate a normal diet without recurrence of disease during 4-24 month follow-up. No other significant complications were reported. ECM scaffold degradation was noted to occur *in vivo* rapidly without any evidence of residual xenogeneic tissue. Using a similar approach, three additional patients were subjected to endoscopic, circumferential *en bloc* resection of Barrett's with HGD, followed by fundoplication.[16] One patient with incomplete ECM coverage developed an easily dilated stricture, whereas the remaining 2 patients who had complete ECM coverage did not. To date, no randomized clinical trials exist comparing the use of ECM in humans following EMR vs. the current standard of care (i.e., observation).

1.5 Study Rationale and Risk Analysis (Risks to Benefits Ratio)

1.5.1 Study Rationale

Data for alternative strategies to prevent stricture formation remain equivocal. The application of ECM, however, appears promising based on preclinical and clinical data, and ECM are FDA approved for use in wound healing. No randomized clinical trials exist.

As an initial step towards assessing the utility of ECM in stricture prevention, we seek to assess the role of ECM in promoting constructive healing following endoscopic resection of esophageal lesions. This will be determined by the ability of a lesion to "lift" with saline injection during routine endoscopic follow-up. The importance of lift was first reported by Uno et al. in colonic lesions.[17] Inability to lift with fluid injection is suggestive of submucosal tumor invasion, but a false-positive nonlift sign may occur after biopsy as a result of the ensuing inflammatory healing process. We hypothesize that the use of ECM in patients undergoing EMR will mitigate this inflammatory process and promote a more constructive model of healing with reformation of the esophageal wall layers as evidenced by a lift sign. Patients receiving ECM will be compared to patients undergoing EMR without application of ECM.

Data from this study may be used to support larger studies investigating the role of ECM in preventing stricture formation in patients undergoing more extensive endoscopic resections via EMR or ESD.

1.5.2 Anticipated Risks and Benefits

The study adds minimal risks to procedures that patients are already scheduled to undergo (EMR). A potential risk of ECM is injury during application to the resection site. We propose the use of endoscopic clips or X-tac to carefully attach each corner of the square ECM material to healthy esophageal tissue surrounding the defect site. This will be carried out by an endoscopist with expertise in esophageal disease and endoscopic therapy. The potential benefit of this includes mitigation of the ensuing inflammatory process. Patients undergoing endoscopic resection return for routine endoscopic follow-up in 8-12 weeks. Injection with saline at this time is safe and will be performed by an experienced endoscopist. Saline is typically resorbed by the tissue within minutes of injection.

1.6 Anticipated Duration of the Clinical Investigation

Screening and enrollment will be performed at the time of consent for their endoscopy and anticipated resection. In eligible patients randomized to ECM placement, the endoscopy will require an additional 5-10 minutes for application of ECM. Follow-up will occur per standard of care in all patients undergoing EMR, that is endoscopy in 8-12 weeks or sooner for patient symptoms (e.g., dysphagia).

2 Study Objectives

2.1 Primary Objective

To assess the efficacy of ECM products in promoting constructive healing as determined by the ability of the resection site to lift during follow-up

2.2 Secondary Objective

To assess the efficacy of ECM products in promoting constructive healing as determined by endoscopic inspection via white light and electron chromoendoscopy.

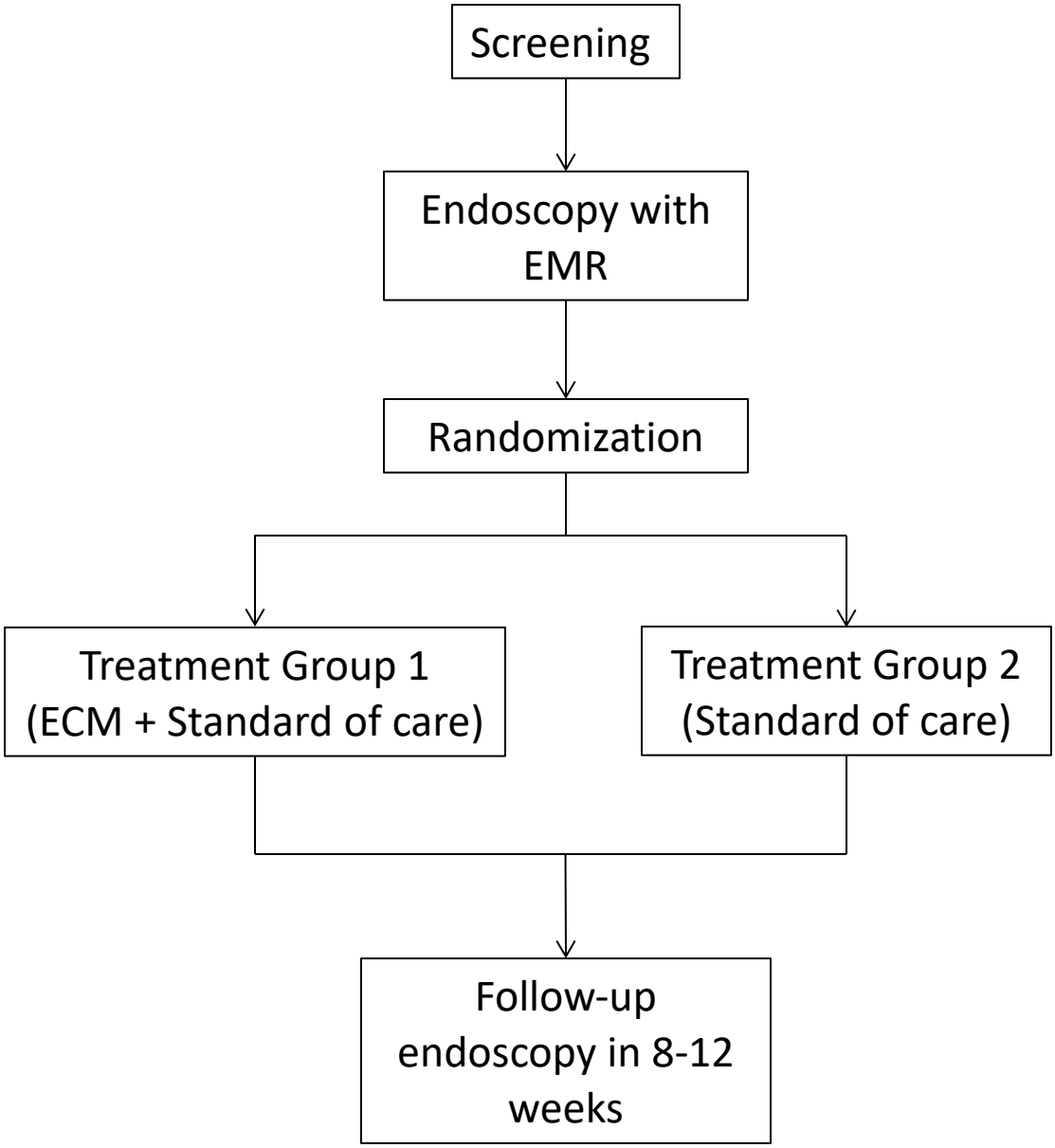
3 Study Design

3.1 General Design

This is a randomized study on the efficacy of ECM in promoting constructive healing following endoscopic resection (EMR). 20 patients anticipated to undergo endoscopic resection will be recruited in the outpatient setting from the Barrett's Esophagus Unit. Prior to endoscopy, patients will complete a standardized questionnaire (Mayo Dysphagia Questionnaire) and the physician will use the Mellow-Pinkas scale to assess baseline dysphagia. Patients with lesions that lift and are amenable to cap-assisted EMR will be randomized to receive ECM to the defect site vs. standard of care. All patients should be on standard of care double dose proton-pump inhibitor therapy (Omeprazole 40mg PO daily or the equivalent); if this has not been initiated they will be provided a prescription .

In both groups, patients will be brought back for repeat standard of care clinic and endoscopic assessments in 12-16 weeks, or sooner if needed. A clinical dysphagia assessment will be performed with repeat of the above questionnaires. Endoscopy will involve inspection of the wound site, and images in white-light and electron chromoendoscopy will be taken for blinded evaluation later. Submucosal saline injection at the wound site will be performed to determine its ability to lift.

Any patients receiving esophageal dilations will be noted. Patients requiring esophagectomy will be excluded from the primary objective of the study but their surgical specimen at the site of endoscopic resection will be evaluated for evidence of constructive healing.



3.2 Primary Study Endpoints

The primary endpoint will be efficacy of ECM in promoting mucosal lift with fluid injection after healing from prior EMR.

3.3 Secondary Study Endpoints

Secondary endpoints include efficacy of ECM in preventing symptomatic esophageal stricture formation requiring dilation, preventing dysphagia as determined by questionnaires, and mitigating formation of post-resection scarring as determined by blinded comparison of

pre- and post-resection images (taken using white-light endoscopy and electron chromoendoscopy).

3.4 Primary Safety Endpoints

Primary safety endpoints include overall survival, incidence of all serious adverse events including unanticipated adverse device effects, incidence of technical and device failures and malfunctions, and quality of life as measured by the above dysphagia questionnaires.

4 Subject Selection, Enrollment and Withdrawal

Patients will be recruited from the Barrett's Esophagus clinic where patients with Barrett's esophagus, esophageal cancer, and/or esophageal nodules are referred for consideration of endoscopic evaluation and management. Patients with advanced esophageal cancer are typically not referred as they warrant chemoradiotherapy or palliative care, and are not candidates for endoscopic management. Thus, this clinic presents a selective population to screen from, and one we anticipate will be able to provide the proposed number of subjects for this study. It is estimated 75-100 patients with esophageal lesions undergoing EMR will be screened to ultimately meet study enrollment goals.

4.1 Inclusion Criteria

- 18 and older
- Male or female
- Able to provide consent
- Scheduled for standard of care endoscopy that may require initial EMR evaluation of esophageal lesions
- Histological evidence of intestinal metaplasia with dysplasia or intramucosal carcinoma

4.2 Exclusion Criteria

- Pregnant women
- Age less than 18
- Prior esophageal EMR or ESD in the same region
- Anyone unable to provide informed consent
- Medical co-morbidities precluding EGD evaluation
- History of chemoradiotherapy to the neck/esophagus
- Unable to stop anticoagulation therapy (non-steroid anti-inflammatory medications are permissible)

4.3 Subject Recruitment, Enrollment and Screening

Subjects will be recruited from the endoscopic practice of the Principal Investigator and Co-Investigator. Patients will undergo screening for inclusion and exclusion criteria at the time of clinical consultation.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects will be withdrawn from the study if any adverse events related to EMR, or application of ECM occurs, including major bleeding, perforation, or infection. Patients will also be withdrawn if after EMR the patient proceeds to esophagectomy.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

An attempt will be made to collect follow-up data for withdrawn subjects, as well as patients who undergo esophageal surgery (i.e., surgical histology of the resection site). These patients will be called to collect this data.

5 Study Device

5.1 Description

ECM products such as those produced by Acell are approved by the FDA to aid in the healing of wound sites like those created by EMR. The product that will be used in this study is the A-cell Matristem, which is derived from porcine bladder (also known as urinary bladder matrix). Observations pertaining to this specific product have been reported in several clinical series, including use in patients undergoing esophageal surgery and hiatal hernia repair.[18-20]

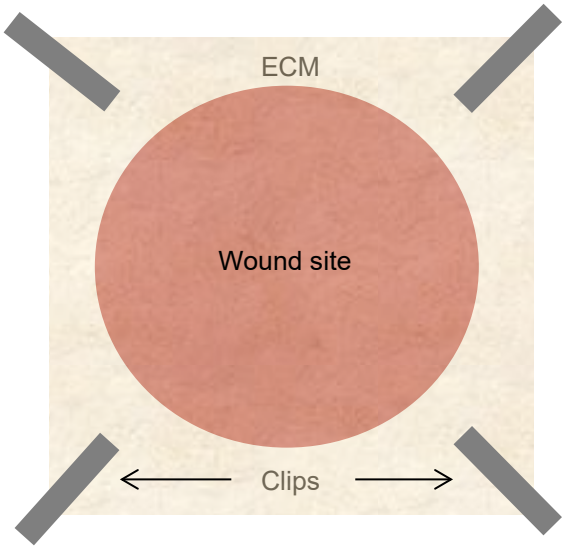
The device is supplied in sheets of varying sizes and thickness. Only one thickness will be used for all patients, whereas the smallest sized sheet that adequately covers the defect site will be selected and applied by the endoscopist. Identifying product information including serial, lot, and/or batch numbers will be recorded for each patient.

5.2 Method for Assigning Subjects to Treatment Groups

Patients will be randomized using prepackaged envelopes to ECM or standard of care and stratified based on the size and number of EMR performed during the procedure. Thus, randomization will occur during the procedure immediately following resection.

5.3 Preparation and Administration/Implantation of Investigational Device

The smallest sized version of material that adequately covers the defect site will be selected for application. Application will involve attachment of the corners of the material to healthy tissue surrounding the defect site using four standard endoscopic clips as depicted below. These clips are designed to slough following healing and rarely cause nuisance or complications to patients. X-tac is also a similar method but using screws instead.



5.4 Subject Compliance Monitoring

Per endoscopic resection protocol, patients will be scheduled for follow-up in 12-16 weeks for repeat endoscopic assessment. Aside from continued use or initiation of proton pump inhibitor therapy (which will be assessed by patient history) there are no interim monitoring requirements for the patient. Patients lost to follow-up or referred for esophageal surgery will be excluded from analysis for the primary objective.

5.5 Prior and Concomitant Therapy

No additional medications or therapies will be provided during this study beyond omeprazole 40mg once daily or the equivalent which is recommended in all patients undergoing endoscopic resection. All prior or concomitant medical therapies can be continued during this study.

5.6 Packaging and Labeling

The ECM product will arrive in a sterile, airtight sleeve. Each sleeve will be inside of a sealed box.

5.7 Masking/Blinding of Study

Patients will be randomized during the procedure based on our current understanding of risk factors for post-resection scarring and stricture formation. Thus, randomization will be primarily determined by the size of the defect and number of resections performed (during the initial endoscopy).

5.8 Receiving, Storage, Distribution and Return

5.8.1 Receipt of Investigational Devices

The ECM product is already stocked and stored in our lab located in the SMH Alfred Main endoscopy suite. Additional inventory will similarly be shipped directly from the manufacturer. At the initiation of the study, investigators will count and verify current inventory. This will be repeated whenever inventory is expanded.

5.8.2 Storage

The manufacturer instructions recommend storage in a clean, dry environment at a temperature of 15 – 35 C. Our laboratory temperature will be maintained within this range. Storage in our laboratory will also prevent unintended/unauthorized use. The product is sterilized and once opened must be used.

5.8.3 Distribution of Study Device

For patients randomized to receive ECM, the ECM will be prepared in the endoscopy suite for application. This will include notation of the manufacturer, catalog number, batch code, and serial number, in addition to details regarding the procedure (including patient information, procedure date and time). A running total number of devices in inventory will be maintained. Any products inadvertently damaged or not used will be noted. Inventory audits will be performed on a monthly basis.

5.8.4 Return or Destruction of Study Device

We will order clinical supply as needed and dispose of unused supply. We do not anticipate needing to return supplies.

6 Study Procedures

6.1 Visit 1

Patients will be recruited from the Barrett's Esophagus Clinic. Patients referred for endoscopic evaluation of Barrett's esophagus with high grade dysplasia , esophageal nodules or early esophageal cancer and are scheduled for EMR, will be screened using inclusion and exclusion criteria outlined above. Patients meeting inclusion criteria will be consented for study inclusion and will be asked to complete the Mayo Dysphagia Questionnaire.

6.2 Visit 2

Visit 2 refers to the procedure during which the patient will undergo planned EMR of the esophageal lesion. Visits 1&2 may take place on the same date. Patients will undergo endoscopy and those amenable to standard EMR will be randomized to receive ECM or receive standard of care (i.e., observation). ECM will be applied to patients in the manner described above.

6.3 Visit 3

All patients will be scheduled to return in approximately 12-16 weeks (up to 6 months) following EMR for follow-up endoscopic evaluation. Patients will repeat the Mayo Dysphagia Questionnaire prior to endoscopy. During endoscopy, the EMR site will be inspected and multiple pictures will be taken in white-light as well as electron chromoendoscopy (i.e., narrow band imaging). Thereafter, standard submucosal saline injection of the healed EMR site will be performed, and the endoscopist will record whether no lift, a partial lift, or complete lift was observed. Symptomatic structuring will be treated with standard pneumatic dilation.

Schedule of Events			
Study Activity	**Baseline***	**Procedure***	**Follow-up procedure (3-4 months)**
Consent	X		
History	X		X
Physical Exam	X	X	X
Disease State Symptom Survey	X		X
Concurrent Medications	X	X	X
Adverse Events		X	X
Serious Adverse Events		X	X

***Baseline and Procedure may be on the same date/visit.**

7 Statistical Plan

7.1 Sample Size Determination

A power analysis was performed for this two independent sample study with a dichotomous endpoint (presence of a saline lift during follow-up EGD). We assume 20% of patients receiving standard of care and no ECM will have a submucosal lift vs. 80% of patients receiving ECM will have submucosal lift. Using a power of 0.8, and alpha of 0.05, this equates to a sample size of 10 for each group for a total of 20 subjects.

7.2 Statistical Methods

Descriptive Statistics

Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for the treatment groups. These analyses will help identify potential confounding variables to be used as covariates in sensitivity analyses. Distributions across subgroups used in randomization will be compared to assess whether the randomization was successful in equalizing distributions of these prognostic variables across treatment groups. Putative prognostic variables that will be investigated through these descriptive analyses include variables such as presence of submucosal lift, esophageal caliber, need for esophageal dilation, and score on the Mayo Dysphagia Questionnaire.

Handling of Missing Data

Given the small number of patients and minimal follow-up time we do not anticipate missing data.

Primary Hypothesis: To assess the efficacy of ECM products in promoting constructive healing as determined by the ability of the resection site to lift with saline injection during follow-up.

A student unpaired t-test will be performed to compare the incidence of successful lift in the area of prior EMR among ECM and non-ECM groups.

Secondary Hypothesis: Secondary endpoints include efficacy of ECM in preventing symptomatic esophageal stricture formation requiring dilation, preventing dysphagia as determined by questionnaires, and mitigating formation of post-resection scarring as determined by blinded comparison of pre- and post-resection images (taken using white-light endoscopy and electron chromoendoscopy).

Dysphagia questionnaire: student paired t-test will compare the pre and post treatment scores between both groups.
Esophageal diameter: student paired t-test will compare the pre and post treatment sizes between groups.

Interim Analysis

As this is a small pilot study, we will not perform an interim analysis. Any serious adverse events will prompt immediate reevaluation of study methods.

7.3 Subject Population(s) for Analysis

All-completed population: Only subjects who completed ALL study related procedures and follow-up will be included (Per Protocol) maybe adjusted to subjects who completed the majority of the study visits and procedures. Patients lost to follow-up will be accounted for during calculation of the attrition rate.

8 Safety and Adverse Events

All adverse events occurring during the study, including those not meeting the criteria of an Unanticipated Adverse Device Effect (UADE) will be recorded on the appropriate case report form. Records of these events will be maintained and reports submitted to the FDA and IRB according to the regulatory requirements. Expected clinical adverse events and nonsignificant (not serious) clinical adverse events will not be reported. Expected clinical adverse events and anticipated adverse device effects are those listed in Section 1.5.2.

8.1 Definitions

Unanticipated Adverse Device Effect (UADE)
A UADE is any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device if that effect, problem or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Adverse Effect (Event)
Any untoward medical occurrence in a subject involved in clinical study of an investigational device; regardless of the causal relationship of the problem with the device or, if applicable, other study related treatment(s).

Associated with the investigational device: There is a reasonable possibility that the adverse effect may have been caused by the investigational device.

Life-threatening adverse effect: Any adverse effect that places the subject, in the view of the investigator, at immediate risk of death from the effect **as it occurred**. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse effect: An adverse effect is considered "serious" if, in the view ofthe investigator, it results in any of the following outcomes:

- death
- a life-threatening AE
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect.

Unanticipated adverse effect: Any adverse effect, the nature, specificity, severity, or frequency of which is not consistent with the risk information in the clinical study protocol or elsewhere in the current IDE application.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as an unanticipated adverse device effect unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the local investigator should instruct each subject to report, to the local investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The local investigator should notify the Mayo IRB of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The IRB should also be notified if the local investigator should become aware of the development of problems, cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets all of the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, **AND**
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as *90* days following the last administration of study treatment.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Study subjects will be routinely questioned about adverse effects at study visits. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. All observed or volunteered adverse effects (serious or non-serious) and abnormal test findings, regardless of the treatment group if applicable or suspected causal relationship to the investigational device or if applicable other study treatment or diagnostic product(s) will be recorded in the subjects' case history. For all adverse effects sufficient information will be pursued and or obtained as to permit; an adequate determination of the outcome, an assessment of the casual relationship between the adverse effect and the investigational device or, if applicable other study treatment or diagnostic product. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

Causality and severity assessment
The investigator will promptly review documented adverse effects and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse effect; 2) if there is a reasonable possibility that the adverse effect was caused by the investigational device or other study treatments; and 3) if the adverse effect meets the criteria for a serious adverse effect.
If the investigator's final determination of causality is "unknown and of questionable relationship to the investigational device or other study treatments," the adverse effect will be classified as associated with the use of the investigational device or other study treatments for reporting purposes. If the investigator's final determination of causality is "unknown but not related to the investigational device or other study treatments," this determination and the rationale for the determination will be documented in the respective subject's case history.

8.3 Investigator Reporting of Unanticipated Adverse Device Effects and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The investigator will evaluate the event and determine the necessary follow-up and reporting required.

The investigator will promptly review documented Unanticipated Adverse Device Effects and as necessary shall report the results of such evaluation to FDA within 10 working days and Mayo IRB within 5 working days of initial notice of the effect. Thereafter the sponsor-investigator will submit such additional reports concerning the effect as requested.

8.3.1 Investigator Reporting, Notifying Mayo IRB

The investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Information collected on the adverse event worksheet (*and entered in the research database)*:

- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research drug*:
- If the adverse event was expected:
- The severity of the adverse event: **
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

8.3.2 Investigator Reporting: Notifying the FDA

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The investigator will report to the FDA all unanticipated adverse device effects according to the required reporting timelines, formats and regulations.

The investigator will submit a completed FDA Form 3500A to the FDA's Center for Devices and Radiological Health for any observed or reported adverse effect that is determined to be an unanticipated adverse device effect. A copy of this completed form will be provided to the DSMB and all participating sub-investigators.

The completed FDA Form 3500A will be submitted to the FDA as soon as possible and, in no event, later than 10 working days after the investigator first receives notice of the adverse effect.

If the results of the investigator's follow-up evaluation shows that an adverse effect that was initially determined to not constitute an unanticipated adverse device effect does, in fact, meet the requirements for reporting; the investigator will submit a completed FDA Form 3500A as soon as possible, but in no event later than 10 working days, after the determination was made.

For each submitted FDA Form 3500A, the investigator will identify all previously submitted reports that that addressed a similar adverse effect experience and will provide an analysis of the significance of newly reported adverse effect in light of any previous, similar report(s).

Subsequent to the initial submission of a completed FDA Form 3500A, the investigator will submit additional information concerning the reported adverse effect as requested by the FDA.

Reporting Process
Unanticipated Adverse Device Effect reports will be submitted on FDA Form 3500A. The contact information for submitting reports is:

Food and Drug Administration
Center for Devices and Radiological Health
Document Mail Center - WO66-G609
10903 New Hampshire Avenue
Silver Spring, Maryland 20993-0002

Deviations from the investigational plan.

The investigator shall notify Mayo IRB (see 21 CFR 56.108(a) (3) and (4)) of any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency. Such notice shall be given as soon as possible, but in no event later than 5 working days after the emergency occurred. Except in such an emergency, prior approval by the investigator is required for changes in or deviations from a plan, and if these changes or deviations may affect the scientific soundness of the plan or the rights, safety, or welfare of human subjects, FDA and IRB notification in accordance with 21 CFR 812.35(a) also is required.

8.4 Unblinding Procedures (Breaking the Blind) (as necessary if the study is blinded)

Unblinding will occur for any serious adverse event related to the ECM, or if execution of other therapies such as esophageal surgery, will be impacted by knowledge of the presence of ECM.

8.5 Stopping Rules

Should an adverse event occur in any subject, further enrollment would be stopped immediately.

8.6 Medical Monitoring

It is the responsibility of the investigator to oversee the safety of the study. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see Section 10 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data comprise all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. When applicable, information recorded on the CRF shall match the Source Data recorded on the Source Documents.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not obliterate, erase, or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction.

Data Management

Data will be collected onto the CRF immediately during visits. It will then be rapidly de-identified and transferred to a secure computer based data base.

Data Processing

Data will be processed using electronic data management programs.

Data Security and Confidentiality

CRF will be kept in a lock drawer in a locked room at the St. Mary's campus. Electronic data will be de-identified, with the coding information for each patient kept on the CRF. Electronic data will be maintained on a secure server within encrypted data files.

Data Quality Assurance

Given the low number of patients, once all data has been collected, all CRF will be audited by an independent study team member to ensure that data transferred to the electronic database is accurate.

Data Clarification Process

Questions about data recording will be resolved by the principle investigator. Notation of the question and its resolution will be made on the CRF and in the electronic database.

9.4 Records Retention

The investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The investigator will retain the specified records and reports for:

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717, whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the monitor, and government regulatory agencies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

Participation as the investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review

and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed and dated by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study will be funding through existing, internal sources.

13 Publication Plan

There are no publication requirements for this pilot study. Decision to publish will be at the discretion of Dr. Kenneth Wang, the principle investigator.

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