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In-bag enzymatic splenic digestion: a novel alternative to manual morcellation?



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ABSTRACT

Background: Contained in-bag spleen morcellation is a conventional extraction technique for safe spleen removal during laparoscopic splenectomy. Existing data for the use of in-bag enzymatic splenic digestion as an alternative to morcellation are lacking. This proof-of-concept study sought to evaluate the effectiveness of single and combinatorial enzyme digestion of murine spleens.

Materials and methods: Murine spleens were digested with collagenase alone or with combinations of commercially available enzymes (collagenase, elastase, hyaluronidase, neutral protease) to determine their degradation effect. The primary end point was the percentage of mass reduction at 15 and 30 min.

Results: For collagenase alone ($n = 15$), the mean reduction in mass was $14 \pm 10\%$ (range: 2%-31%) at 15 min and $30 \pm 25\%$ (range: 7%-100%) at 30 min. Using combinatorial dissolution with collagenase, hyaluronidase, and elastase ($n = 8$), the mean reduction in mass was $27 \pm 16\%$ (range: 6%-42%) at 15 min and $48 \pm 27\%$ (range: 3%-100%) at 30 min. Injecting the enzyme solution into whole spleens ($n = 9$) yielded a mean reduction in mass of $22 \pm 13\%$ (range: 9%-42%) at 15 min and $55 \pm 31\%$ (range: 9%-100%) at 30 min; mean reduction was $9 \pm 13\%$ (range: 0%-39%) at 15 min and $23 \pm 13\%$ (range: 3%-53%) with no injection ($n = 12$).

Conclusions: We provide the first demonstration of successful enzymatic murine spleen digestion as an alternative method for in-bag spleen removal during laparoscopic splenectomy. However, the significant cost and quantities of commercial enzyme required for clinical application dampens the enthusiasm for this novel approach.

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Introduction

Splenectomy is frequently performed via minimally invasive surgery (MIS) for various, primarily hematologic, indications in children and adolescents. One limitation of using MIS techniques during splenectomy is the difficulty of safely extracting specimens through small incisions. The most conventional method for spleen withdrawal is a contained extracorporeal in-bag morcellation approach whereby the specimen is placed within an Endo Catch bag and removed from the peritoneal cavity after manual morcellation. Massive splenomegaly (spleen weight ≥ 500 g) precludes this approach due to the maximum capacity of the Endo Catch bag.¹ Other described bag-related complications include perforation and deployment failure, with a perforation rate of up to 4%.¹ Bag perforation has been reported to lead to the spillage of splenic fragments within the abdominal cavity² and even to splenosis and immune thrombocytopenic purpura recurrence after therapeutic splenectomy.³ Alternative splenic extraction techniques include specimen fragmentation in the abdominal cavity (risk of splenosis), extended trocar incision, mini-laparotomy, Pfannenstiel incision^{4,5} or natural orifice specimen extraction in adult females.⁶

Although these methods allow for specimen removal with a minimally invasive approach, some authors have argued that the advantages of laparoscopy are not clear in patients with massive splenomegaly, thereby generating concerns about the safety of this minimal access surgery in children with massive splenomegaly.^{7,8} Nevertheless, recent work from Hassan and Ali supports the feasibility and safety of laparoscopic splenectomy for pediatric hematologic disorders, and these authors, among others, consider the MIS approach to be the gold standard regardless of spleen size.^{9–12} Given the technical challenges associated with retrieval of large specimens during laparoscopy, our query was whether a targeted in-bag spleen enzyme digestion method would present an alternative option to facilitate specimen removal.

The spleen is known for its complex immune cell repertoire and can also be characterized by its unique extracellular matrix (ECM). Studies performed on the ECM of the spleen show two structurally and functionally distinct types: the interstitial matrix and the basement membrane. The molecular composition of both the interstitial matrix (collagen types I, II, and III, fibronectin, tenascin-C) and basement membrane (laminins, collagen type IV, proteoglycans, nidogen) has been well described and reviewed elsewhere.¹³ Because the main splenic structures (capsule, trabeculae, vascular walls, and reticular fibers) contain ECM components, we theorized that digesting collagen and other components within the spleen ECM with collagenase and other ECM-directed enzymes may have value as an extracorporeal in-bag “biologic morcellator” to aid with specimen retrieval. This concept has not been studied with the spleen, but translational work using *Clostridium histolyticum* collagenase (CHC) has evaluated highly purified CHC as a nonhormonal local treatment for diseases characterized by a high content of collagen that can be effectively digested and result in reduced tissue stiffness. Described clinical uses for CHC include uterine leiomyomas (fibroids)^{14,15} and multiple fibrotic diseases, including

Dupuytren’s contracture,^{16,17} keloid scars,^{18,19} and Peyronie’s disease.^{20–22} Currently, CHC is approved by the Food and Drug Administration only for Dupuytren’s contracture and Peyronie’s disease.

The objective of this study was to investigate whether commercially available enzymes can effectively digest the ECM in murine spleen specimens and thereby evaluate the feasibility of developing splenic enzymatic digestion into an alternative specimen extraction technique for laparoscopic splenectomy. This proof-of-principle study would provide a basis for further clinical evaluation.

Materials and methods

Animals

C57BL/6 mice were maintained under barrier conditions, and their spleens were harvested and used for enzymatic digestion. Studies were carried out at St. Jude Children’s Research Hospital (SJCRH), and the protocol was reviewed and approved by the SJCRH Institutional Animal Care and Use Committee. A total of 30 spleens were treated and analyzed in the experiments.

Materials

The following enzymes were purchased from Worthington Biochemical Corp. (Lakewood, NJ): type V collagenase (CLS-5; C), hyaluronidase elastase, and neutral protease/dispase. For reference, the 1-g bottle of CLS-5 had assayed enzyme activities of 550 U/mg dw collagenase, 570 U/mg dw caseinase, 2.32 U/mg dw clostripain, and 0.10 U/mg dw tryptic acid. Hyaluronidase activity was 770 U/mg dw, and elastase activity was assayed at 4.41 U/mg dw. Candidate enzymes were chosen based on previous methods used to degrade connective tissues and allow for tissue dissolution and subsequent cell isolation.^{23–32} Hank’s balanced salt solution plus Ca^{2+} and Mg^{2+} (Gibco; Grand Island, NY) was used as the basic digestion medium. The solution-based conditions are similar to those used in previous protocols directed toward spleen dissolution for the purpose of cell isolation.^{23–32} Each spleen or spleen section was incubated in buffer solution before digestion to ensure osmotic equilibration before mass and volume evaluation.

Single and combination solution-based enzymatic digestion of murine spleens

Murine spleens were sectioned in half and weighed to 0.0001-g precision on a laboratory scale. The average dry mass of the halves was 0.0459 ± 0.00873 g (range: 0.0327–0.0684 g). Weighed mass had good consistency among the halves. The specimens were then equilibrated in digestion buffer and subjected to single or combination digestion with CLS-5 (C), hyaluronidase (H), elastase (E), and neutral protease (NP) used in different combinations and concentrations. [Appendix Tables A1–A2](#) show the enzyme combinations, concentrations, and masses used in the study. The combinations were

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