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# Viscoelastic properties of human pancreatic tumors and *in vitro* constructs to mimic mechanical properties

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is almost universally fatal, in large part due to a protective fibrotic barrier generated by tumor-associated stromal (TAS) cells. This barrier is thought to promote cancer cell survival and confounds attempts to develop effective therapies. We present a 3D *in vitro* system that replicates the mechanical properties of the PDAC microenvironment, representing an invaluable tool for understanding the biology of the disease. Mesoscale indentation quantified viscoelastic metrics of resected malignant tumors, inflamed chronic pancreatitis regions, and histologically normal tissue. Both pancreatitis (2.15 ± 0.41 kPa, Mean ± SD) and tumors (5.46 ± 3.18 kPa) exhibit higher Steady-State Modulus (SSM) than normal tissue (1.06 ± 0.25 kPa; $p < .005$). The average viscosity of pancreatitis samples (63.2 ± 26.7 kPa·s) is significantly lower than that of both normal tissue (252 ± 134 kPa·s) and tumors (349 ± 222 kPa·s; $p < .005$). To mimic this remodeling behavior, PDAC and TAS cells were isolated from human PDAC tumors. Conditioned medium from PDAC cells was used to culture TAS-embedded collagen hydrogels. After 7 days, TAS-embedded gels in control medium reached SSM (1.45 ± 0.12 kPa) near normal pancreas, while gels maintained with conditioned medium achieved higher SSM (3.38 ± 0.146 kPa) consistent with tumors. Taken together, we have demonstrated an *in vitro* system that recapitulates *in vivo* stiffening of PDAC tumors. In addition, our quantification of viscoelastic properties suggests that elastography algorithms incorporating viscosity may be able to more accurately distinguish between pancreatic cancer and pancreatitis.

**Statement of Significance**

Understanding tumor-stroma crosstalk in pancreatic ductal adenocarcinoma (PDAC) is challenged by a lack of stroma-mimicking model systems. To design appropriate models, pancreatic tissue must be characterized with a method capable of evaluating *in vitro* models as well.

**Statement of Significance:** Our indentation-based characterization tool quantified the distinct viscoelastic signatures of inflamed resections from pancreatitis, tumors from PDAC, and otherwise normal tissue to inform development of mechanically appropriate engineered tissues and scaffolds. We also made progress toward a 3D *in vitro* system that recapitulates mechanical properties of tumors. Our *in vitro* model of stromal cells in collagen and complementary characterization system can be used to investigate mechanisms of cancer-stroma crosstalk in PDAC and to propose and test innovative therapies.



## 1. Introduction

Pancreatic cancer is diagnosed in about 50,000 cases annually and claims nearly 45,000 lives annually. The 5-year relative survival is 8%, and late diagnosis in more than half of the cases reduces 5-year survival to 3% [1]. Pancreatic ductal adenocarcinoma (PDAC)

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comprises most cases of primary pancreatic tumors, and PDAC tumors are characterized by dense regions of fibrosis surrounding clusters of cancer cells. The physical properties of these desmoplastic tumors have important, wide-ranging implications for both diagnosing and treating PDAC. The fibrosis and stiffening associated with PDAC tumors may be detectable through minimally-invasive methods like elastography and leveraged for diagnosis [2,3]. However, stiffening of the pancreas is also expected to accompany the chronic inflammation of pancreatitis, as in other inflammatory gastrointestinal diseases [4,5], which may confound diagnosis. Direct mechanical characterization of normal, PDAC, and pancreatitis tissue could inform development of non-invasive diagnosis.

Mechanical properties of the PDAC microenvironment would also inform scientific investigations of PDAC tumor growth and metastasis and translational development of therapies. PDAC is typically diagnosed in advanced stages once tumors have already developed dense desmoplasia, and the fibrotic matrix and reduced vasculature impedes delivery of chemotherapeutic agents [6,7]. Though dense fibrotic stroma may act as a barrier for diffusivity of drug agents, the deletion or suppression of stromal cells in tumors has been shown to accelerate cancer progression *in vivo* [8–10]. In addition, stiffness of the tumor microenvironment in other cancers has been shown to contribute to cancer cell proliferation [11] and chemoresistance [12,13]. A better understanding of tumor tissue mechanics may improve 3D *in vitro* models of tumors for mechanistic investigations and for development and screening of therapies.

To inform both clinical practice and the development of *in vitro* models, we collected surgical specimens from human patients for direct mechanical characterization using a custom indentation system. By comparing normal, PDAC, and pancreatitis samples, we show that both elastic stiffness and viscous relaxation properties are required to distinguish between PDAC and pancreatitis. To demonstrate that *in vitro* microenvironments can be tuned to have similar properties, we characterize Living Mechanical MicroEnvironments (LiMMEs) for direct comparison to patient samples.

Our custom equipment ensured identical methods and analysis could be used on both patient samples and *in vitro* hydrogels. Comparing across methods and constitutive models can result in dramatically different values to describe stiffness. Various methods of characterizing collagen hydrogels, for example, have resulted in values for mechanical properties that span many orders of magnitude (Fig. 1) [14–22]. For comparison, reported shear storage moduli were converted to elastic-like moduli assuming Poisson's ratio is 0.495 [23]. While collagen concentration and stiffness generally rise together, the potential for inconsistency in soft matter characterization across *ex vivo* and *in vitro* samples is evident. The ability of our system to quantify mechanical properties for synthetic hydrogels and tissue resections alike provides us with the advantage of direct comparison, and this work calls attention to nuances of mechanical characterization in addition to its biological findings.

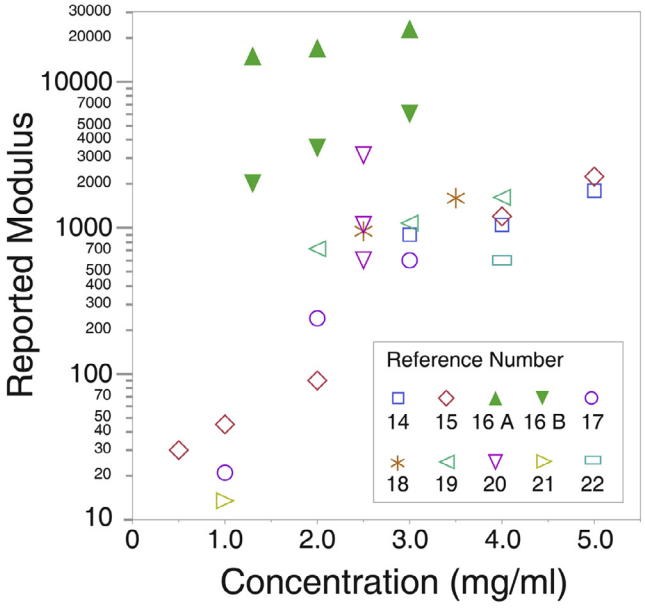


**Fig. 1.** Reported moduli for collagen gels range three orders of magnitude for small concentration variations. Different papers reporting modulus to denote elastic component of mechanical properties use various names, e.g. Young's Modulus, Elastic Modulus, Apparent Modulus, and Storage Modulus, and cover wide-ranging values for similar concentrations and formulations. Precise test conditions are described in respective reports [14–22], but notably, effective modulus is different at low (0.1 mm/min, 16A) and high (1 mm/min, 16B) displacement rates [16]. For comparison, reported shear storage moduli were converted to elastic-like moduli assuming Poisson's ratio is 0.495 [23].

## 2. Materials and methods

### 2.1. Specimen preparation

#### 2.1.1. Patient samples

Resected pancreatic tissue was generously donated by the Department of Surgery after obtaining appropriate patient consent under University of Florida Institutional Review Board-approved protocols. Tissue was collected from surgeries between 10/2014 and 02/2017. Before each surgery that was expected to result in in a pancreatic tumor, indentation equipment was prepared. After surgery, a portion of the resected tissue went to pathology and to satisfy other clinical requirements. If enough tissue was left, the remaining portion was prepared for indentation testing. PDAC samples were excluded from study if patients had received chemo- or radiation therapy prior to surgery. "Normal" tissue with no histologic evidence of pancreatitis or malignancy was only available accompanying other conditions such as benign cystic lesions or duodenal adenomas. Taken together, these constraints severely limited samples available for study.

Resected tissue samples were placed in DMEM/F12-10% FBS culture media on ice in the operating room and transported to laboratory for testing. The size of the sample varied with each patient, but generally volumes ranged between 125 and 600 $mm^3$. If the sample had not already been sectioned with flat, parallel surfaces, the sample was sliced in a stainless-steel matrix slicer (Zivic Instruments) to obtain a flat indentation surface approximately 2 mm thick (Fig. 2A). Samples were loosely confined in a custom holder made of silicone (Fig. 2B) and submerged with room temperature culture media (Fig. 2C). Tissue was allowed to reach ambient temperature before indentation, and all indentations were carried out no more than 2 h after resection.

#### 2.1.2. Collagen gels

10× DMEM (Sigma) was combined in a 1:1 ratio with HEPES (Gibco) to obtain a 5× DMEM and HEPES solution. 3.7 g of sodium bicarbonate (Fisher Bioreagents) per 160 mL was added to control pH. Rat tail collagen type-I (Corning) was diluted with 0.2% acetic acid (Glacial, Fisher Scientific) in 1× Phosphate Buffer Saline (PBS) (InvitroGen, ThermoFisher) to obtain a 4 mg/mL collagen solution. 5× DMEM was warmed to 37 °C and mixed with the collagen solution at 4 °C in a 1:3 ratio to obtain a 3 mg/mL collagen hydrogel precursor solution. Collagen was then allowed to thermogel at 37 °C for 35 min before adding cell culture medium.

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