POLITECNICO MILANO 1863

SCUOLA DI INGEGNERIA INDUSTRIALE
E DELL'INFORMAZIONE

Executive Summary of the Thesis

Development of a test protocol for the experimental characterization of the mechanical and adhesive properties of sealants for pancreatic surgery

Laurea Magistrale in Biomedical Engineering - Ingegneria Biomedica

Authors: Matteo Natale, Lorenzo Picerno

Advisor: Prof. Maria Laura Costantino

Co-advisors: Prof. Francesco De Gaetano, Prof. Francesco Cellesi, Dr. Arianna Callera

Academic year: 2022-2023

1. Introduction

The pancreas represents one of the fundamental glands in the human body, performing both exocrine and endocrine functions to facilitate and enhance digestive system activity. This gland can be subject to various pathologies, both non-neoplastic such as pancreatitis and diabetes, and neoplastic like pancreatic cancer, which constitutes the most lethal tumor of the digestive system with an effective 5-year survival rate of 9% [5].

Surgical interventions like pancreaticoduodenectomy contribute to improving treatment outcomes; however, the most frequent complication is pancreatic fistula (POPF). The main challenge associated with POPF is the lack of in-depth studies, implying the absence of devices specifically designed for this procedure.

A pancreatic fistula involves the leakage of a volume of fluid, with amylase levels three times higher than the normal serum amylase activity limit, associated with a clinically relevant condition. This phenomenon occurs in 22-26% of cases and can lead to various additional infectious, clinical, or surgical complications.

After pancreaticoduodenectomy, sutures are commonly used but may fail in pancreatic reconstruction due to the soft and fragile consistency of the pancreas. Additionally, the pancreas is fixed in the retroperitoneal space; thus, intestinal movement can induce anastomotic rupture. Furthermore, the pancreas's deep location and proximity to many vital organs and vessels make pancreatic surgery more challenging and risky.

To overcome these challenges, a collaboration between the Humanitas Clinical Institute and the Politecnico of Milan was established with the aim of developing a method that could completely or partially replace the use of sutures. This approach involves not only the combined use of adhesive and sutures but ideally, the use of sealants specific to pancreatic tissue alone.

Historically, Ethibloc (Ethicon, Norderstedt, Germany) adhesive was one of the earliest solutions, but its inconsistent long-term efficacy was attributed to its viscosity [4, 9]. TachoSil (Corza Health, San Diego, USA), a fibrin-based adhesive, was effective for various organs but posed a risk of postoperative fistulas in pancreatic surgery [7]. Dermabond (Ethicon, Inc., Somerville, NJ, USA), composed of 2-

octylcyanoacrylate (2-OCA), adapted to pancreatic physiology but could lead to long-term postoperative fistula formation [11]. Neoprene showed promise in reducing pancreatic fistula rates, albeit with a higher incidence of postoperative diabetes [8, 12]. Glubran2 glue (GEM s.r.l., Viareggio, Italy) holds promise, pending comprehensive studies on its properties [6]. BioGlue (CryoLife, Inc., Kennesaw, USA) is extensively studied for reducing postoperative pancreatic fistulas, despite potential systemic inflammation due to released compounds interacting with immune cells [1, 10].

2. Aim of the thesis work

The mechanical characterization of sealants used in pancreatic surgery still represents a gap in the scientific literature, as there is no standardized protocol for this purpose. Therefore, the main objective of this work is to devise a specific procedure.

This procedure involved the use of tissue segments taken from pig pancreases, joined together using surgical adhesives and glues available in the medical field. Swine pancreas is used due to its mechanical characteristics similar to those of humans.

In this way, it was possible to analyze and evaluate the adhesive strength for each glue and adhesive through uniaxial tensile and lap-shear tests. For this purpose, ASTM standards F2258-05 and F2255-05 were employed, with some modifications to adapt them to the scope of this project.

The primary aim of this study is to create polymeric grips capable of accommodating pancreas samples for the proper execution of the proposed characterization protocol.

3. Materials and methods

The biological tissue used in this study consists of pancreas harvested from male pigs at 11 months of age at the time of slaughter. Each tissue sample was provided by the same slaughterhouse, and once removed from the carcass, the pancreas was frozen at -20 degrees Celsius. Twenty-four hours before each test, the portion of tissue to be used was thawed in a refrigerator at 6 degrees Celsius, while the remaining portion was returned to the freezer, awaiting the next test. Before cutting the samples for mechanical tests, each tissue required the removal of adipose tissue, fibrotic tissue, and organs other than the pancreas.

Within this thesis work, surgical adhesives such as TachoSil (Corza Health, San Diego, USA), Hemopatch (Baxter Healthcare, Zurich, Switzerland), Tabotamp Hemostatic (Ethicon SARL, Neuchâtel, Switzerland), and Tabotamp Fibrillar (Ethicon, Inc., San Lorenzo, Puerto Rico), as well as surgical glues like BioGlue (CryoLife, Inc., Kennesaw, USA), cyanoacrylate-based glue (Super Attak, Loctite Corporation, Düsseldorf, Germany), Tisseel (Baxter Healthcare, Zurich, Switzerland), and Floseal (Baxter Healthcare, Zurich, Switzerland), were characterized. For each of them, three uniaxial tensile tests and three lap-shear tests were conducted.

In order to perform the two different types of mechanical tests, polymeric grips were obtained through the Form 3B+ 3D printer (Formlab, Massachusetts, US). These allow for the creation of two different experimental setups that evaluate the average adhesive strength of surgical adhesives and glues, calculated as the ratio between the maximum force reached during the test and the initially measured bond area.

The MTS Synergie 200H (MTS System Corporation, Eden Prairie, MN, USA) tensile testing machine, equipped with a 100 N load cell, was used for conducting the tests. Prior to each test, biological samples were cut using a scalpel at room temperature, with a size that varied depending on the specific test, i.e., the useful contact area between the polymeric specimens. To do this, a component of the printed polymeric grips was used as a guide each time, and by following its perimeter, an attempt was made to cut the samples with a useful area as comparable as possible. In order to test the adhesion strength of surgical adhesives or glues, it is necessary to securely attach the biological samples to the polymeric supports. To achieve this, a cyanoacrylate-based glue (Super Attak, Loctite Corporation, Düsseldorf, Germany) was chosen, applied between the pancreatic tissue sample and the surface of the polymeric grip. To facilitate the bonding, a layer of Parafilm was placed on the upper surface of the polymeric grip, and a weight around 60 grams was applied for a duration of 5 minutes.

Each uniaxial tensile and lap-shear test was conducted following the "ASTM F2258-05: Standard test method for strength properties of tissue adhesives in tension" and "ASTM F2255-05: Standard test method for strength properties of tissue adhesives in lap-shear by tension loading" standards, with some modifications tailored to the objectives of this project:

- application of a tightening torque of 20 Nm using a torque wrench for the insertion of the polymeric grips into the test bench;
- an initial compression of -0.5 N is applied in the uniaxial tensile tests; this was not feasible in the lap-shear tests due to the nature of the experimental setup;
- adhesion time of 5 minutes to allow bonding between the biological tissue and the glue or adhesive (except for Floseal glue, which requires an adhesion time of 10 minutes);
- actuator movement speed set at 5 mm/min;
- sampling frequency of the testing machine set at 10 Hz;
- automatic test termination upon reaching an actuator displacement of 10 cm;
- choice if humid or dry environment depending on the chemical polymerization process associated with each glue or adhesive.

In particular, with the exception of Tisseel and BioGlue, all sealants were evaluated in a humid environment. In this latter case, the biological samples were immersed in a physiological solution for 1 minute before each test; during the tests, they were kept moist using a spray containing the same physiological solution.

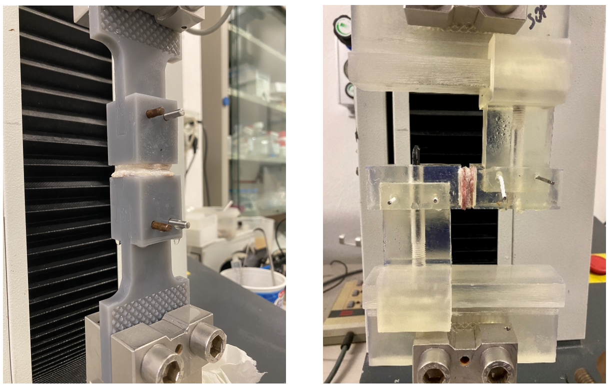

Figure 1: Experimental set-up for carrying out uniaxial tensile (left) and lap-shear (right) tests.

4. Results and Discussions

For each test, the testing machine provided adhesive force values in relation to the measured actuator displacement; these values were valuable in calculating the adhesive stress related to the initially measured useful bond area at the beginning of the test. Three uniaxial tensile tests and three lap-shear tests were conducted for each type of adhesive and glue.

Below are the results associated with the uniaxial tensile tests performed on surgical adhesives in terms of measured force and stress for each test. The adhesive stress is calculated based on the maximum force achieved during each test.

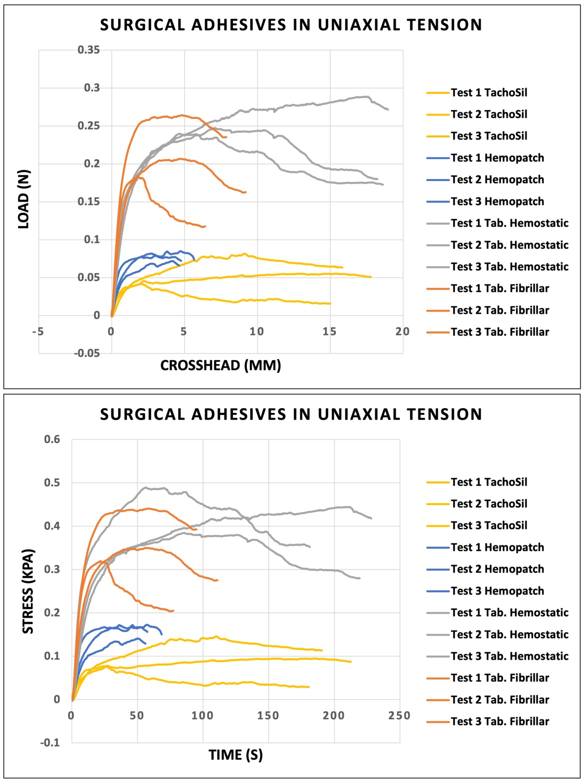

Figure 2: Load-crosshead and stress-time graphs associated with uniaxial tensile tests on surgical adhesives.

The average adhesive stress values obtained during the uniaxial tensile tests on TachoSil, Hemopatch, Tabotamp Hemostatic, and Tabotamp Fibrillar are 0.10 ± 0.04 kPa, 0.16 ± 0.02 kPa, 0.44 ± 0.05 kPa, and 0.37 ± 0.06 kPa, respectively.

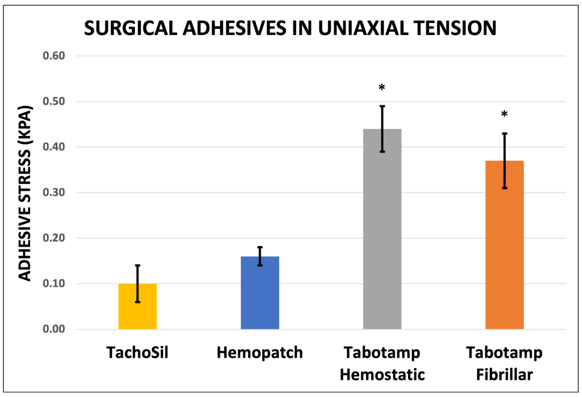

Figure 3: Histogram of the average adhesive stress calculated for each surgical adhesive subjected to uniaxial tensile testing. (* $p < 0.001$)

Following a statistical analysis, post-hoc tests with Bonferroni correction of the ANOVA test revealed that there were no significant differences in adhesive stress values between TachoSil and Hemopatch ($p > 0.05$), and between Tabotamp Fibrillar and Tabotamp Hemostatic ($p > 0.05$). However, Tabotamp Hemostatic and Tabotamp Fibrillar showed significant differences compared to TachoSil and Hemopatch ($p < 0.001$).
TachoSil exhibited the lowest average adhesive stress, presumably due to the absence of blood during the tests, which may have reduced the action of thrombin on fibrinogen contained within the adhesive. Tabotamp Hemostatic achieved the highest average adhesive stress, this can be attributed to: the oxidation and regeneration process of alfacellulose, which optimizes its properties through a uniform structure [2]. Secondly, the reduction of local pH caused by the adhesive [3], which promotes tissue necrosis and the formation of an artificial clot through the absorption of biological fluids and ions. Finally, the precise geometry of Tabotamp Hemostatic adhesive, unique among the four adhesives tested, ensures a uniform distribution on the biological tissue surface, ensuring greater strength. Tabotamp Hemostatic and Tabotamp Fibrillar showed similar average adhesive stresses, likely because of their similar cellulose-based compositions.
Similarly, the adhesive properties of surgical glues were also assessed through uniaxial tensile tests, yielding the following results.

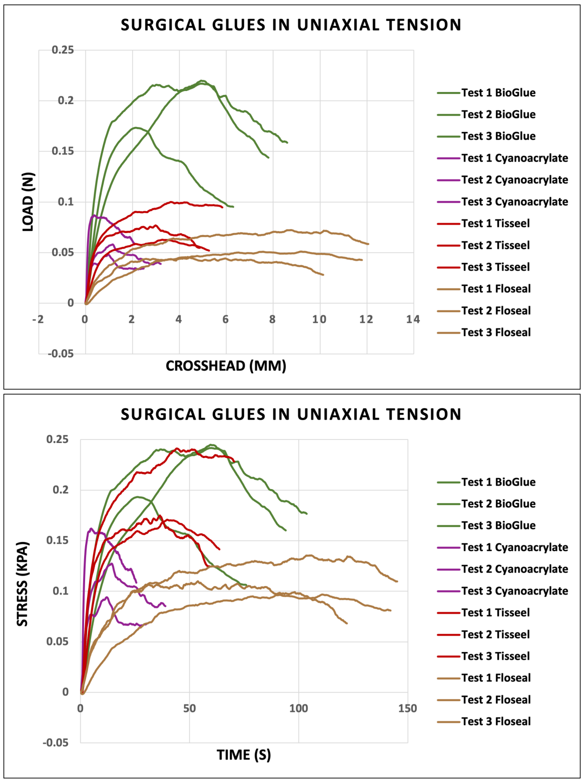

Figure 4: Load-crosshead and stress-time graphs associated with uniaxial tensile tests on surgical glues.

The average adhesive stress values obtained during the uniaxial tensile tests on BioGlue, cyanoacrylate-based glue, Tisseel, and Floseal are 0.24 ± 0.04 kPa, 0.13 ± 0.04 kPa, 0.19 ± 0.04 kPa, and 0.11 ± 0.02 kPa, respectively.

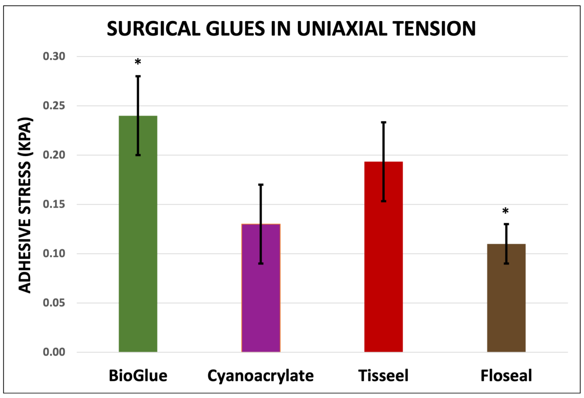

Figure 5: Histogram of the average adhesive stress calculated for each surgical glue subjected to uniaxial tensile testing. (* $p < 0.05$)

As highlighted in Figure 5 , BioGlue exhibits the highest average adhesive stress. It is capable of forming an adhesive matrix compatible with biological tissues through a chemical cross-linking reaction. This reaction forms covalent bonds between glutaraldehyde and albumin proteins, creating a three-dimensional structure that enables it to adhere to tissues. On the contrary, Floseal adhesive shows lower average adhesive stress values because, in the absence of blood, the thrombin present in it cannot convert fibrinogen into fibrin to stabilize tissue adhesion.

Furthermore, BioGlue, Cyanoacrylate, and Tisseel glues did not exhibit statistically significant differences in uniaxial tensile tests following ANOVA testing with Bonferroni correction ($p > 0.05$). BioGlue adheres due to its adhesive matrix, Tisseel forms a clot similar to the one physiologically formed during blood coagulation, and cyanoacrylate forms a solid adhesive barrier that penetrates even into the spaces between tissues. Despite their different compositions, all these adhesives show comparable average adhesive stress thanks to the stability of the chemical bonds formed between the adhesives and biological tissues. Below, the results associated with the lap-shear tests conducted on surgical adhesives are presented in terms of measured force and stress for each test.

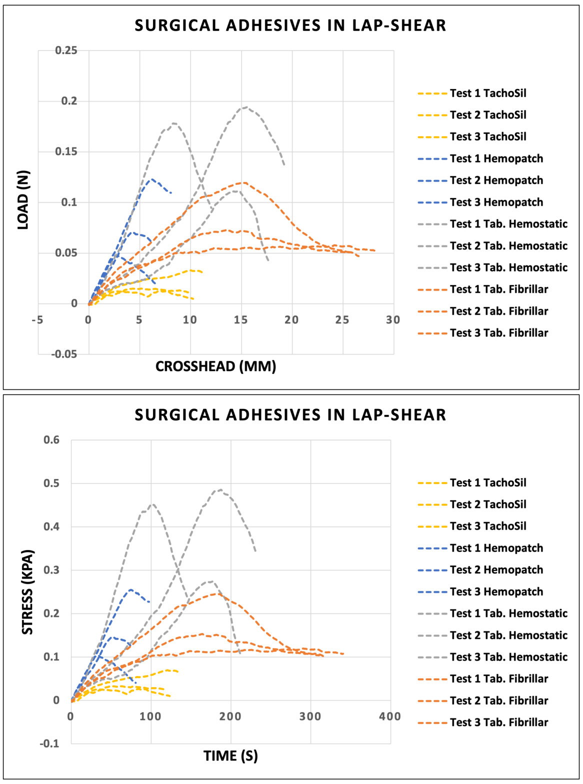

Figure 6: Load-crosshead and stress-time graphs associated with lap-shear tests on surgical adhesives.

The average adhesive stress values obtained through the lap-shear tests on TachoSil, Hemopatch, Tabotamp Hemostatic, and Tabotamp Fibrillar are 0.04 ± 0.02 kPa, 0.17 ± 0.07 kPa, 0.40 ± 0.11 kPa, and 0.17 ± 0.07 kPa, respectively.

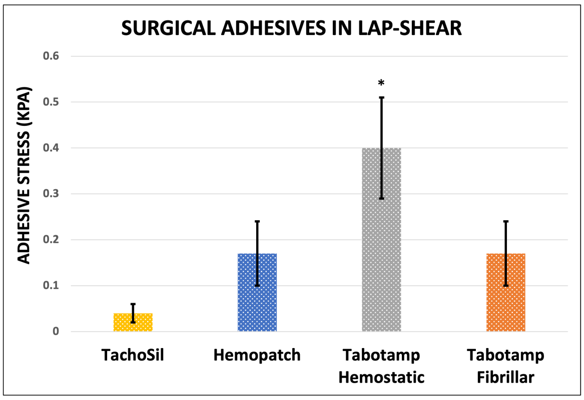

Figure 7: Histogram of the average adhesive stress calculated for each surgical adhesive subjected to lap-shear testing. (* $p < 0.01$)

The average adhesive stress associated with TachoSil appears to be the lowest: once again, this can be attributed to the absence of blood during the tests, which reduces the action of thrombin on fibrinogen contained within the adhesive, making it less effective.

On the other hand, the average adhesive stress associated with Tabotamp Hemostatic is the highest due to: the oxidation and regeneration process of alfacellulose, the formation of an artificial clot and a uniform distribution on the biological tissue surface.

Finally, following ANOVA testing with Bonferroni correction, Tabotamp Fibrillar and Hemopatch did not show statistically significant differences between them ($p > 0.05$). This is related to the fact that both surgical adhesives have a matrix composed mainly of collagen fibers. On the contrary, Tabotamp Hemostatic shows statistically significant differences with each of the other three adhesives ($p < 0.01$).

Similarly, the adhesive properties of surgical glues were also analyzed through lap-shear tests, yielding the following results.

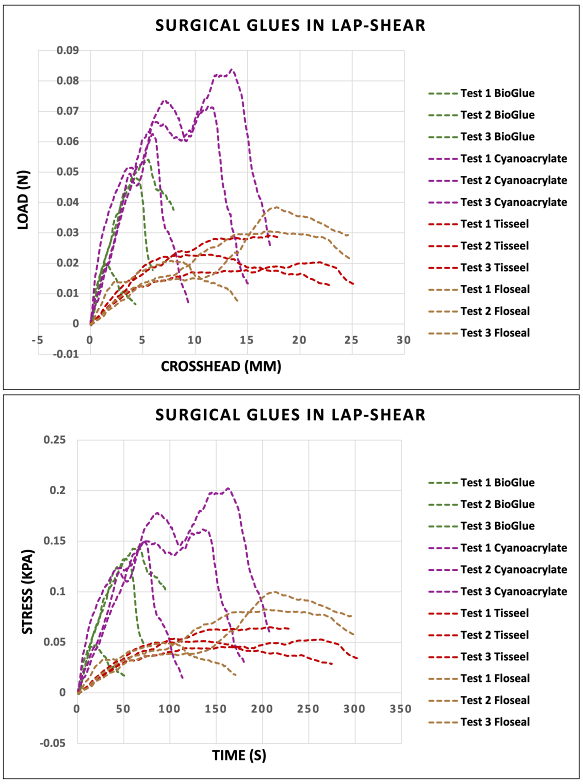

Figure 8: Load-crosshead and stress-time graphs associated with lap-shear tests on surgical glues.

The average adhesive stress values obtained through the lap-shear tests on BioGlue, cyanoacrylate-based glue, Tisseel, and Floseal are 0.11 ± 0.05 kPa, 0.16 ± 0.03 kPa, 0.05 ± 0.02 kPa, and 0.08 ± 0.03 kPa, respectively.

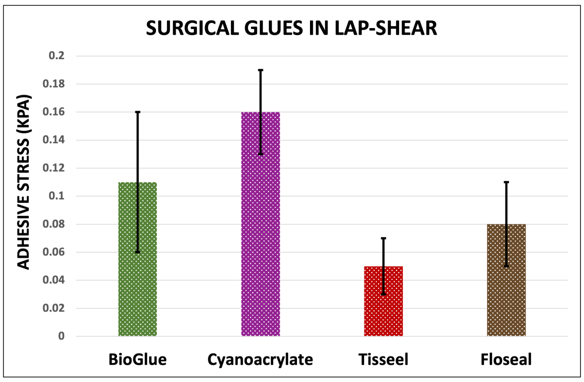

Figure 9: Histogram of the average adhesive stress calculated for each surgical glue subjected to lap-shear testing.

Through statistical analysis, post-hoc tests with Bonferroni correction of the ANOVA test revealed that the differences between the surgical glues are not statistically significant ($p > 0.05$). However, as shown in Figure 9 , it is evident that each glue has an average adhesive stress value with a relatively wide standard deviation. This could be attributed to the lack of repeatability in the initial compression force applied during the adhesion process to biological tissues.

It was expected that Floseal adhesive would have a lower average adhesive stress value compared to other glues since there is no blood present during the tests. This limits the action of thrombin, which normally reacts with fibrinogen in the blood, reducing its ability to convert into fibrin and form stable chemical bonds.

Similarly, the results obtained with Tisseel adhesive are in contrast to expectations. In fact, it was anticipated that Tisseel would show higher adhesive stress values compared to Floseal because it contains fibrinogen. The thrombin present in Tisseel should have converted fibrinogen into fibrin, facilitating the formation of stable chemical bonds.

5. Conclusions and future developments

In conclusion, the study revealed that the uniaxial tensile and lap-shear tests conducted using the defined protocol allow for adequate characterization of sealants used in surgical contexts other than pancreatic surgery. Despite the various limitations encountered in the proposed testing protocol, it represents a fundamental starting point for further research in the field of pancreatic surgery and new adhesive technologies.

To enhance the effectiveness and reliability of the mechanical characterization protocol, it is recommended to implement a more precise method for measuring the quantity of glue used. Additionally, using human pancreatic tissue is suggested to evaluate the differences between human and porcine biological samples.

Likewise, the absence of blood during the experimental tests may have inhibited the formation of chemical bonds between pancreatic tissue and the sealant. Specifically, for the characterized adhesives and Floseal glue, the presence of blood could trigger a coagulation process very similar to the physiological one, increasing their adhesive strength. Therefore, the use of animal-derived blood is suggested during future mechanical tests.

Furthermore, it is advisable to use a load cell with a scale appropriate to the force values ob-

tained.
Lastly, it is essential to develop a test setup for better management and control of compression during the adhesion of biological samples in lap-shear tests. This setup could include an adjustable guide to exert controlled force during bonding.
These efforts could potentially lead to the development of an optimal testing protocol in the future for the mechanical characterization of glues and adhesives specifically designed for pancreatic surgery.

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