Clinical Biomechanics 49 (2017) 34–39

Contents lists available at ScienceDirect

Clinical Biomechanics

journal homepage: www.elsevier.com/locate/clinbiomech

Lecture

# Comparison of mechanical compressive properties of commercial and autologous fibrin glues for tissue engineering applications

Matthew G. Cravens, Anthony W. Behn, Jason L. Dragoo*

*Department of Orthopaedic Surgery, Stanford University School of Medicine, Stanford, CA, USA*

ARTICLE INFO

*Keywords:*
Fibrin
Unconfined compression
Autologous
Tisseel
Evicel

ABSTRACT

*Background:* Fibrin glues are widely used in orthopedic surgery as adhesives and hemostatic agents. We evaluated the compressive properties of selected fibrin glues in order to identify which are appropriate for tissue regeneration applications subject to compression.

*Methods:* Uniaxial unconfined compression tests were performed on fibrin gels prepared from commercial and autologous products: (1) Evicel (Ethicon), (2) Tisseel (Baxter), (3) Angel (Arthrex), and (4) ProPlaz (Biorich). Cyclic loads were applied from 0 to 30% strain for 100 cycles at 0.5 Hz. Following cyclic testing, specimens were subjected to ramp displacement of 1% strain per second to 80% strain.

*Findings:* Throughout cyclic loading, Evicel and Tisseel deformed (shortened) less than Angel at all but one time point, and deformed less than ProPlaz at cycles 10 and 20. The dynamic moduli, peak stress, and strain energy were significantly greater in Tisseel than all other groups. Evicel displayed significantly greater dynamic moduli, peak stress, and strain energy than Angel and ProPlaz. Following cyclic testing, Tisseel and Evicel were significantly less deformed than Angel. No specimens exhibited gross failure during ramp loading to 80% strain. Ramp loading trends mirrored those of cyclic loading.

*Interpretation:* The tested commercial glues were significantly more resistant to compression than the autologous products. The compressive properties of Tisseel were approximately twice those of Evicel. All preparations displayed moduli multiple orders of magnitude less than that of native articular cartilage. We conclude that in knee surgeries requiring fibrin glue to undergo compression of daily activity, commercial products are preferable to autologous preparations from platelet-poor plasma, though both will deform significantly.

## 1. Introduction

Fibrin glues are commonly used in the operating room, but little is known about their ability to withstand compression *in vivo*. Fibrin was first used as a hemostat in 1909, and the first commercial fibrin glue, Tisseel (Baxter Healthcare Corporation, Westlake Village, CA, USA), was approved by the FDA in 1998 (Spotnitz, 2010). Fibrin glue, also called fibrin gel or fibrin sealant, is currently the only material with FDA approval for use as a hemostat, adhesive, and sealant (Spotnitz, 2014).

As the main protein component of a physiologic clot, fibrin is activated from fibrinogen *in vivo* by active thrombin, the end product of the clotting cascade. This forms a soft gel, composed of fibrin monomers assembled into fibrils and eventually fibers in a 3-dimensional network. Factor XIII, if present, can be activated by thrombin to convert the non-covalent bonds between fibrin monomers to covalent bonds, forming a stiffer gel that is less easily digested by plasmin (Sierra, 1993).

Commercial fibrin glues take advantage of a simplified method of fibrinogen activation, bypassing the clotting cascade. Products use a dual syringe applicator to combine a fibrinogen solution with a thrombin-$CaCl_2$ solution. Prior to the FDA approval of commercial glues, surgeons utilized plasma to obtain fibrinogen, which they could combine with bovine thrombin (Spotnitz, 2010). If a fibrin gel is made from the patient's own blood, it is called an autologous fibrin gel.

Today, autologous gels are commonly made from platelet rich plasma (PRP) by combining PRP with purified human thrombin (Nather et al., 2012). The current use of PRP (though frequently not in gel form) in regenerative orthopedics is pervasive (Salamanna et al., 2015). A commonly discarded product of PRP creation is platelet poor plasma (PPP), which has been established as an acceptable source of clotting proteins for fibrin glues (Man et al., 2001). In order to focus solely on the properties of fibrin gels without the presence of platelets, we chose to test autologous products that use PPP, rather than PRP, as a fibrin source.

* Corresponding author at: Department of Orthopaedic Surgery, Stanford University, Stanford University Medical Center Outpatient Center, 450 Broadway Street, Redwood City, CA 94063, USA.
*E-mail address:* jdragoo@stanford.edu (J.L. Dragoo).

http://dx.doi.org/10.1016/j.clinbiomech.2017.08.004
Received 3 June 2016; Accepted 14 August 2017

The goal of this study is to evaluate the compressive properties of various commercial and autologous fibrin glue preparations to identify which are appropriate for *in vivo* tissue regeneration applications that are subject to compression. To our knowledge, no other studies have examined the elastic moduli of these products in unconfined compression testing. We expected to find greater resistance to compression in commercial preparations, simply due to the much higher concentrations of fibrinogen in commercial products compared to autologous.

## 2. Methods

### 2.1. Design

This was an *in vitro* study examining the compressive properties of 5 mm discs of commercial and autologous fibrin glues.

### 2.2. Variables

Commercial and autologous glues were chosen based on United States Food and Drug Administration approval for clinical use and the ability of the physical product to undergo unconfined compression testing.

### 2.3. Specimen preparation

The commercial products, Tisseel (Baxter) and Evicel (Ethicon, Somerville, NJ, USA), produced from pooled plasma with respective fibrinogen concentrations of 67–106 and 55–85 mg/mL (Ethicon, 2009; Baxter Healthcare Corporation, 2013), were prepared according to manufacturer instructions. For autologous product preparation, a whole blood sample was obtained from one donor and partitioned for use in Angel (Arthrex Inc., Naples, FL, USA) and ProPlaz (Biorich Medical, Irvine, CA, USA) systems. For the Angel system, 5 mL sodium citrate was drawn into a 60 mL syringe, followed by 35 mL whole blood. The syringe was injected into the self-contained Angel centrifuge system, and 20 mL platelet poor plasma (PPP) was collected. For ProPlaz, 10 mL sodium citrate was drawn into a 60 mL syringe, followed by 50 mL whole blood. An Emcyte Executive Series centrifuge (Emcyte Corporation, Fort Myers, FL, USA) and a Pure PRP system (Biorich Medical, Irvine, CA, USA) were used to produce PPP. The ProPlaz product was then used to remove water from the PPP by pushing the sample repetitively across a filter, until the PPP was reduced to 2/3 its original volume, resulting in 10 mL of "PPP concentrate." Each autologous product was then gelled by combining 500 IU human thrombin (Thrombin 22-H, Creative Biomart, Shirley, NY, USA) in 40 mM $CaCl_2$ solution. For both Angel and ProPlaz, an Evicel applicator was used to draw PPP into a 10 mL syringe, which was then combined with 1 mL of thrombin solution to make the autologous fibrin gel.

Gels were prepared in Petri dishes and allowed to incubate for 5 min at 25 °C. They were then transferred to a rubber surface, and a 6 mm biopsy punch was used to create cylindrical samples for testing (Diameter: mean 5.1 (SD 0.6) mm, Height: mean 2.2 (SD 0.8) mm). Five replicates were prepared for each group. Specimens were kept in phosphate buffered saline (PBS) at room temperature prior to testing to prevent dehydration; time from specimen production to testing did not exceed 10 min.

### 2.4. Biomechanical testing

Unconfined compression tests were conducted using a 5944 materials testing system (Instron Corporation, Norwood, MA, USA) fitted with a 10 N load cell (Interface Inc., Scottsdale, AZ, USA). The test setup incorporated aluminum compression platens lined with Teflon to minimize friction (Fig. 1). All tests were performed in PBS maintained at 37 °C. Specimen diameter was measured using digital calipers. Height was recorded with the specimen under a preload of approximately 2 mN using the material testing system's position readout (position accuracy = ± 0.02 mm). Specimens were subjected to compressive loading from 0 to 30% strain at 0.5 Hz for 100 cycles. The upper limit of 30% strain was selected to mimic the strains imparted on knee articular cartilage during daily activity (Bingham et al., 2008; Liu et al., 2010; Van de Velde et al., 2009; Yang et al., 2010). Following cyclic testing, specimens were left uncompressed for a 30 s recovery period prior to being loaded at a rate of 1% strain/s to a maximum of 80% strain. Load and displacement data were recorded at 100 Hz.

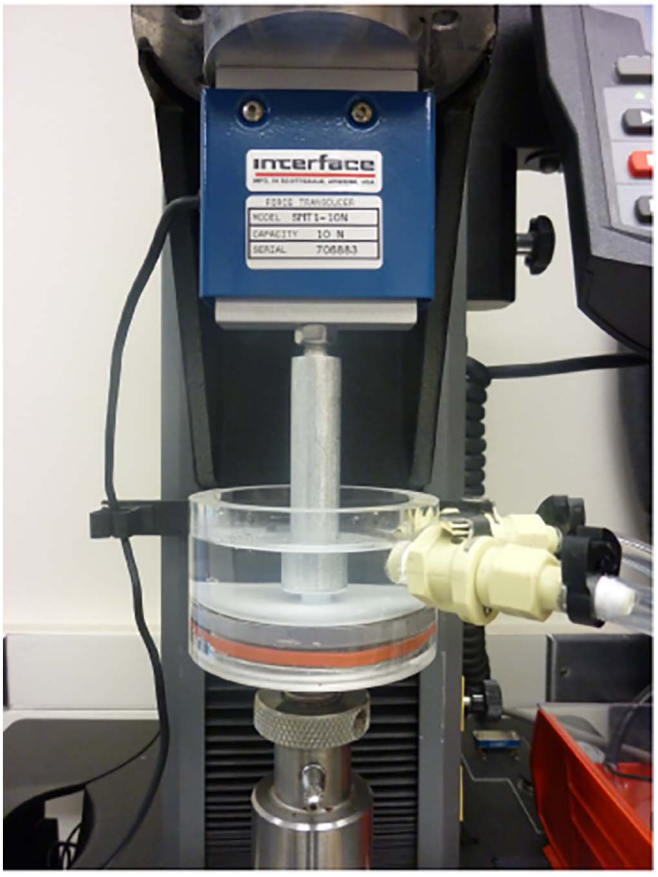


**Fig. 1.** Experimental setup for unconfined compression tests. Fibrin gels were submerged in a PBS bath maintained at 37 °C.

Displacement and load data were corrected for the test setup compliance and buoyancy force on the upper compressive platen, respectively. The inelastic response of the materials was characterized for each loading cycle by measuring the displacement of the machine actuator prior to the load output reaching the initial preload. Cyclic deformation, which served as a measure of specimen shortening, was defined at the amount of displacement required to reach the initial preload divided by the original specimen height. The dynamic modulus was determined from a linear curve fit of the stress *versus* strain loading curve from 20 to 30% strain. Loading strain energy was defined as the area under the stress *versus* strain curve. Peak stress was also recorded for each loading cycle.

The post cyclic testing deformation following the 30-second recovery period, modulus in the range 20–30% strain, stress at 80% strain, and strain energy to 80% strain were calculated from the ramp load test data. Material failure was defined as a drop in the load displacement curve during the single ramp test to 80% strain.

### 2.5. Statistical analysis

Groups were compared using one way analysis of variance (ANOVA)