Evaluation of Absorbable Surgical Sealants: In vitro Testing

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SUMMARY: Three absorbable in situ setting sealing products were evaluated (DuraSeal™, CoSeal™ and HemaSeel® Fibrin Sealant) for preparation time, mechanical strength, water uptake, and burst pressure strength. Testing was performed immediately after preparation, and after soaking in 37°C PBS for 1, 2 and 3 days. All three commercially available absorbable surgical sealants demonstrate reductions in material and mechanical characteristics when exposed to a simulated physiologic environment over a period of 3 days. When comparing energy to failure in a physiologic environment for 1, 2 and 3 days, DuraSeal averaged 5 times stronger than CoSeal, and 3 times stronger than Fibrin Sealant.

INTRODUCTION
The need for an effective, tissue compatible absorbable sealant for sutured closure augmentation is widely recognized. When used in conjunction with sutures, in situ setting sealant technologies should allow for the creation of leak-free closures. In order to function adequately, suture line sealants must have tissue adherence and internal cohesive strength adequate to withstand fluid leaks through needle holes. This adherence and strength must be maintained after surgery to continue sealing while the suture line heals naturally underneath.

This report compares three different absorbable suture line sealants for practical and functional characteristics. Specifically, features such as time of preparation and sealant reaction time are compared. Also, acute and sub-acute strength, percent increase in volume due to water take-up and bursting strength are evaluated.

MATERIALS AND METHODS
Three commercially available in situ setting absorbable sealants were obtained for bench top evaluation: (a) DuraSeal (Confluent Surgical, Waltham, MA), (b) CoSeal (Angiotech Pharmaceuticals, Vancouver, BC), and (c) HemaSeel Fibrin Sealant (Haemacure Corporation, Sarasota, FL, manufactured by Baxter Healthcare Corp). DuraSeal and CoSeal are two-part self-curing synthetic hydrogel products, while HemaSeel is a two-part self-curing fibrin sealant. Reconstitution of the sealants results in two liquids, or precursors, that rapidly react without external energy sources when mixed during application to form a solid clot (fibrin sealant) or hydrogel (DuraSeal and CoSeal). In the testing described here, all products were applied and set under ambient surgical conditions. Storage and preparation of each product was performed according to manufacturer Instructions for Use.

Sample Preparation
The time required by a trained individual to prepare each product per Instructions for Use was recorded.

Sealant Reaction Rate
A rapid sealant reaction rate is critical to avoid excess run-off when applied to non-horizontal surfaces. Sealant reaction rate was determined by adding 100µL of the first precursor to a test tube containing a stir bar (7x2 mm, VWR, West Chester, PA) rotating at 300 rpm. Then, 100µL of the second precursor was added to the test tube. The time between addition of the second precursor and a stop in stir bar rotation was measured with a stopwatch. This time represents the reaction rate of the precursors, and was measured immediately and 1 hour after preparation.

Mechanical Testing
The rapid liquid-to-solid reaction rates of these products makes the creation of conventional “dog bone” samples for tensile testing difficult. Thus, for this testing, both liquids were rapidly injected through a static mixer (TAH Industries, Robbinsville, NJ) and into 6.35 mm ID silicone tubing (Nalgene, Rochester, NY). This tubing was then cut into 6.35 mm lengths, from which cylindrical sealant plugs were removed.

These cylindrical samples were compression tested at time zero, and after 1, 2 and 3 days in 37°C PBS. Mechanical testing included (a) Compressive elastic modulus (E, kPa) and (b) Energy to Failure in Compression (N•mm). Uniaxial compressive mechanical tests were conducted at ambient room temperature and humidity using a screw driven load frame (Model 4442 Instron, Instron Corporation, Canton, MA). One preconditioning cycle of 0.1 N load was followed by a final loading ramp to compressive failure at a rate of 25.4 mm/sec. All load displacement data was digitally collected and analyzed.
Sealant Water Uptake
Sealant plugs were weighed immediately after creation, and after soaking for 1, 2, and 3 days in 37°C PBS.

Burst Pressure Testing
Burst pressure testing was performed to determine the strength of the interface bonding between collagenous materials and the sealant (ASTM 2392-04). Collagen casing (#320, Nippi, Inc., Tokyo, Japan) with a uniform 3.0 mm skin biopsy punch defect was used as the tissue substrate onto which each of the sealing products was applied. Sealant thickness was measured with calipers and the sample was either tested immediately, or after 1 day in 37°C PBS. The water pressure at which sealant failure occurred (adhesive or cohesive failure) was recorded as the burst pressure.

RESULTS
Sample Preparation
The average preparation time for the different sealants is shown in Figure 1. Fibrin sealant took the longest to prepare (12.3 ± 2.0 min), followed by CoSeal (3.2 ± 0.0 min) and DuraSeal (1.0 ± 0.0 min). All kits were prepared per the Instructions for Use by trained individuals.

Sealant Reaction Rate
The sealant reaction rate in all instances was shown to occur within less than 3 seconds immediately after mixing. At time zero, fibrin sealant showed a significantly longer sealant reaction time compared to DuraSeal and CoSeal. At 1 hour, fibrin sealant showed a significantly longer sealant reaction time than DuraSeal (Figure 2).

Mechanical Testing
The measured initial elastic modulus of each sealant was seen to decrease with time; however, the CoSeal samples were so degraded that sample handling was difficult at 3 days (Figure 3). Compared to the dramatic changes over 3 days observed for CoSeal (96% reduction), the changes for DuraSeal and fibrin sealant were more moderate.

The sealant Energy to Failure followed the same trends where reductions over the 3-day period were observed from the initial values of 9.7 ± 1.9, 31.3 ± 6.4, and 24.2 ± 13.1 N•mm for fibrin sealant, DuraSeal, and CoSeal respectively (Figure 4). DuraSeal retained the highest Energy to Failure over 3 days as compared to CoSeal and fibrin sealant.
Sealant Water Uptake

The percent change in sealant plug weight can be seen in Figure 5. Over the 3 days, CoSeal increased on average 558% in weight, compared to DuraSeal at 98%, and fibrin sealant at 3%.

![Figure 5: Sealant plug Weight Change.](image)

Burst Pressure Testing

Sealant burst pressure was found to differ significantly for the three sealants evaluated. The average burst pressure, normalized by sealant thickness, can be seen in Figure 6.

![Figure 6: Sealant Average Burst Pressure.](image)

Each burst pressure failure was characterized as either adhesive (failure at the tissue-sealant interface) or cohesive (failure in the sealant material) failure. The higher the rate of cohesive failure, the better the tissue adherence. Figure 7 presents the percentage of samples tested that exhibited a cohesive failure mechanism.

![Figure 7: Rate of Cohesive Failures.](image)

DISCUSSION

Increasing demand for the surgical adoption of in situ setting absorbable sealants to aid in the sealing of resected tissue, incision, or wound closure sites requires a better characterization of the material and mechanical properties of these sealant products in terms of simulated physiological environment (37°C, PBS) over short term time periods (up to 72 hours).

TISSEEL® VH and HemaSeel are the same products marketed by different companies (both manufactured by Baxter Healthcare Corp). Unlike the hydrogel products tested, fibrin sealants are broken down via enzymatic action in the body. Thus, a potential limitation of this study is the 37ºC PBS environment, which contains no fibrinolytic enzymes. Thus, the fibrin sealant data reported here is likely best case, due to limited in vitro degradation. DuraSeal (Figure 8) and CoSeal are synthetic hydrogels that break down in the body due to hydrolysis.

![Figure 8: DuraSeal Sealant applicator.](image)

Although there were differences in the amount of time required to prepare each product, all three materials reacted at similar rates (< 3 seconds). Time for preparation is an important consideration in surgical procedures, since the need for sealants cannot always be anticipated. Also, rapid reaction rate is important to minimize the amount of sealant run off when applied to slanted surfaces.
Large differences were noted in the amount of water uptake. Fibrin sealant water absorption was negligible during the test period. In contrast, DuraSeal and CoSeal both absorbed water while soaking in PBS. However, the amount of DuraSeal water uptake was significantly less than that of CoSeal. At 3 days, DuraSeal uptake was less than 98% compared to 558% for CoSeal. The percent increase in any one axis can be calculated from the volumetric increase. Assuming uniform expansion in all axes, the volumetric swelling at 3 days corresponds to a single axis increase of 87%, 26% and 1% for CoSeal, DuraSeal and fibrin sealant, respectively.

Ideally, the sealant elastic modulus would be similar to the surrounding tissue in order to minimize interfacial stress during tissue movement. The DuraSeal modulus of 42 kPa at time zero compares well to published modulus values for various tissues.

Large differences in sealant strength were also evident in testing. Energy to Failure testing evaluates the sealant cohesive strength, while the burst testing evaluates both the cohesive and adhesive strength. Sealant strength should be maintained long enough for tissues to heal adequately following implantation. The 1-day Energy to Failure and burst strength results, relative to DuraSeal, can be seen in Figure 9. At 1 day, CoSeal Energy to Failure and burst strength was 23% and 16% that of DuraSeal, while fibrin sealant Energy to Failure and burst strength was 41% and 4% that of DuraSeal.

![Figure 9: Strength at 1 day, relative to DuraSeal.](image)

In most potential indications, sealant tissue adherence and persistent strength are critical features for proper function. In the interface burst pressure test, DuraSeal had by far the highest burst pressures, and the lowest failures at the collagen substrate interface. These results suggest higher DuraSeal cohesive strength and better tissue adherence, both critical features of a functional surgical sealant.

**CONCLUSIONS**

Test results from the three products demonstrated large differences. In summary, DuraSeal was found to have the:
- Fastest preparation time
- Fastest reaction rate 1 hour post preparation
- Highest strength (Energy to Failure) out through 3 days
- Moderate water uptake, leading to a 26% dimensional increase at 3 days
- Highest burst strength
- Best tissue adherence during burst testing