Tailoring the Mechanical & Bio-Response Properties of a Natural Tissue Adhesive Using Pharmaceutical Excipients

Orahn Preiss Bloom, Guy Tomer
LifeBond Ltd., Caesarea, Israel

Introduction: Tissue adhesives that are made from natural polymers frequently rely on toxic chemical reagents or to crosslink the polymer chains, resulting in poor biocompatibility. Synthetic adhesives may have better safety profiles but lack the inherent biomimetic characteristics of natural polymers that contribute to biological compatibility and properties such as support of cell ingrowth and tissue healing. Therefore, there is a need to develop a natural polymer based adhesive that will combine the benefits of both types of materials.

Payne et al. initially demonstrated the adhesive properties of gelatin crosslinked with microbial transglutaminase (mTG), wherein the mTG forms a thermally stabilized gelatin adhesive. Gelatin, a derivative of collagen, is a desirable biomaterial as it has been used for decades as a hemostat and has a well established safety record. However, some of the natural properties of gelatin (solid state of gelatin solutions at operating room temperatures, overabundance of active groups in gelatin fibrils) present challenges in making a gelatin-mTG adhesive feasible for clinical use. The current study demonstrates that these natural shortcomings can be overcome through the studied blend of specific approved pharmaceutical excipients. In this manner, the tissue adhesive can be adapted for clinical use without compromising the beneficial mechanical and biomimetic properties of the natural polymer. This study also describes mechanical testing methods and animal experiment models used to test the modified adhesive for use in surgical procedures.

Methods: Adhesive gelatin hydrogels were formed by mixing aliquots of gelatin solution (type A porcine gelatin) with aliquots of enzyme solution (microbially-synthesized transglutaminase - mTG) at a constant volumetric ratio. Various pharmaceutical grade (USP, EP) excipient chemicals were incorporated, as specified.

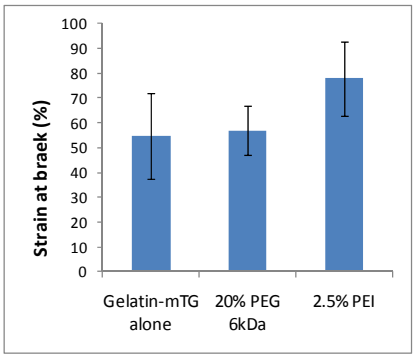
Fig 1: Dogbone shaped gelatin hydrogel before (left) and after (right) tensile test on Instron Material Testing System

The mechanical properties (elastic modulus, strain to break) of the different gelatin-based adhesive hydrogel formulations were tested using an Instron™ Material Testing Sytem. Curing time was measured using a rotating spindle visocometer with t-bar spindle and helipath. Liquid-gel transition point of gelatin solutions was measured using an air bearing rheometer.

Biocompatibility of various formulations was tested *in vitro* in cell cultures and *in vivo* in rabbit implantation.

Ex vivo performance was examined on incised porcine intestinal segments using a burst pressure test. *In vivo* performance was tested on porcine intestinal staple line models and arterial bleeding models.

Results: The transition point of the concentrated gelatin solutions was successfully lowered from 33°C to below 18°C to ensure liquid state of the solutions at operating room temperatures. This was accomplished using primarily urea and $CaCl_2$. The amounts and ratio of these excipients in the gelatin solution were carefully controlled to ensure biocompatibility and no degradation of mechanical properties. To further neutralize the free $CaCl_2$ and maintain the positive biocompatibility profile, sodium citrate was added to the mTG component to act as a calcium chelator in addition to its buffer capacity.



Graph 1: Effect of plasticizers on flexibility of mTG crosslinked gelatin after 2 hours incubation

The crosslinked gelatin hydrogels were very flexible shortly after curing but became more brittle after 2 hours, probably as a result of over-crosslinking resulting from an overabundance of active groups in the gelatin. Such brittleness resulted in reduced performance *in vivo*. Several plasticizing materials were tested to maintain hydrogel flexibility with varying levels of success. While classical plasticizers, such as PEG, were not very effective, the inclusion of 2.5-5.0% polyethyleneimine increased elasticity significantly (Graph 1).

Ex vivo acute efficacy tests demonstrated the differential cohesive and adhesive strength of excipient-modified adhesive formulas in burst pressure models. *In vivo* tests demonstrated successful use of the modified adhesives in sealing staple lines and bleeding wounds in pigs. With proper tuning of excipient additive concentrations and balances, fully biocompatible formulas were achieved.

Conclusions: The current study demonstrates that it is possible to modify the mechanical and bio-response properties of a natural polymer adhesive to meet clinical needs using only approved pharmaceutical excipients. Using the same principles, the gelatin-mTG adhesive can be further tailored for other applications such as cell scaffold, chronic wound care, and localized drug delivery.