A HIGHLY ELASTIC ADHESIVE BASED ON PHOTO-CROSSLINKED GELATIN

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Introduction

We have recently described the use of a ruthenium-based photochemistry, first described by Fancy and Kodadek [1], to form crosslinked protein polymers [2-4]. Certain proteins, among them resilin and fibrinogen, can be covalently crosslinked by formation of di-tyrosine (di-Tyr) bonds in seconds using visible light. A photo-crosslinked fibrinogen hydrogel displayed adhesive strength ~5-fold higher than a commercial fibrin-based adhesive [3]. Here we show that gelatin is also well suited to this rapid photo-chemistry, and can be crosslinked into a material with very high elasticity and tissue adhesive strength.

Experimental

Materials

Porcine type A gelatin, ~300 g Bloom, bovine type B gelatin, ~225 g Bloom and cold water fish gelatin, ~60 kDa were obtained from Sigma. Porcine gelatin was also derivatised using *N*-succinimidyl-3-[4-hydroxy phenyl]propionate (Bolton-Hunter reagent) to increase its Tyr content. Bovine fibrinogen was prepared as previously described [3]. Tris(2,2′-bipyridyl)dichloro-ruthenium(II) hexahydrate (RuTBP) and sodium persulfate (SPS) were from Sigma.

Photo-crosslinking

A photo-crosslinking process [2-4] was used to cast pieces of crosslinked gelatin. Mixtures of gelatin (typically 100-175 mg/mL), 1 mM RuTBP and 20 mM SPS in PBS were dispensed into moulds and irradiated for 30 sec at room temperature with a 600 W quartz tungsten halogen lamp.

Adhesive and tensile testing

Adhesive properties were assessed as previously described [3]. Thus, 125 μL of test solution was placed between 2 pieces of bovine amnion, each stretched over the end of a Perspex cylinder (176 mm^2). The force required to pull the membranes apart, at a rate of 1 mm/min, was measured on an Instron 5544 tester with a 5 N load cell. Tensile properties were analyzed using dumbbell-shaped samples of crosslinked material, with a gauge length of 8 mm and a cross-sectional area of 5 mm^2, at a rate of 5 mm/min failure.

In vivo studies

The efficacy of crosslinked gelatin as a tissue adhesive was tested in a sheep model according to CSIRO and University of Melbourne Animals Ethics protocols. The sheep were anesthetized and a thoracotomy was performed. A 25 mm long, 6 mm deep incision was made into the surface of a lung lobe, then closed with two interrupted sutures (4-0 Vicryl). Approximately 250 μL of gelatin mixture was applied to the closed defect and photo-cured for 45 sec. The seal was examined visually for 15 min before closing the chest to ensure there was no leakage of blood and air. The animals were allowed to recover, and were euthanised after 2 and 4 weeks for examination and histology.

Results and Discussion

Crosslinking of gelatin

Gelatin solutions could be rapidly crosslinked into a gel of defined shape that remained stable for at least 4 h at 90°C, while a control sample of thermally gelled gelatin dissolved completely within 1 min (Fig 1). Amino acid and HPLC analyses showed that in photo-crosslinked gelatin no Tyr was detected, while di-Tyr had been formed leading to cross-linking.

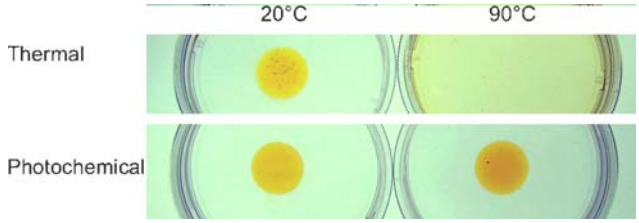

Fig. 1. Thermal stability of crosslinked gelatin.

Adhesive properties

All the gelatins tested adhered strongly to bovine amnion after crosslinking, with values for stress at break approximately up to ~6 fold higher than that of a commercial fibrin tissue sealant (Fig. 2). Photo-crosslinking was complete in only 15 sec compared to 15 min for the commercial, thrombin activated product. For photo-crosslinked materials, the porcine gelatin had the highest adhesion strength (>100kPa), followed by bovine gelatin, bovine fibrinogen and fish gelatin. Adhesive strength varied with different concentrations of reagents, different curing times, and differing light sources. Concentrations of at least 1 mM RuTBP and 10 mM SPS were required for maximum adhesive strength of porcine gelatin (Fig. 2). A curing time of

only 20-30 sec was necessary to achieve maximum adhesive strength when irradiating with a xenon lamp.

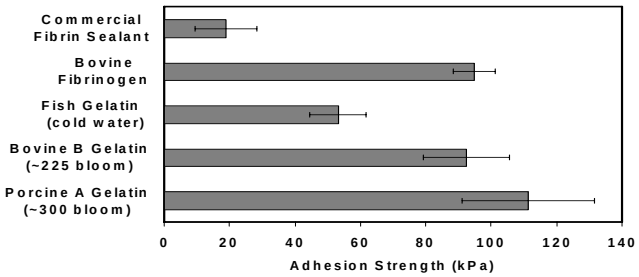

Fig. 2. Adhesive strength of crosslinked materials, comparing photo-crosslinked gelatins with fibrinogen.

Histology showed that gelatin formed a continuous layer, approximately 40 μm thick, that adhered tightly to both amnion surfaces. Fragments from the amnion remained attached to the adhesive layer after it was stretched to break (Fig. 3). In light of the comparatively high tissue adhesive strength of the photo-crosslinked gelatin, it is possible that covalent bonds are formed between the gelatin and the tissue.

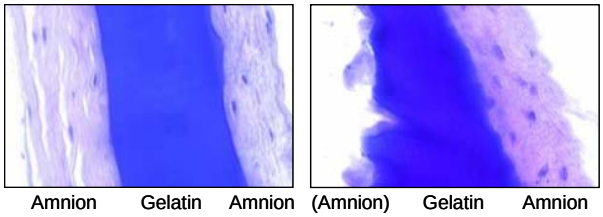

Fig. 3. Crosslinked gelatin adhered to amnion before and after tensile failure.

Tensile properties

Photo-crosslinked porcine gelatin had high ultimate tensile strength (UTS) and very high extension to break (Table 1). This UTS was higher than for bovine gelatin and >8-fold higher than for bovine fibrinogen, while extension to break was equally much higher. Phenolic-derivatised gelatin had up to a 5-fold higher elastic modulus than the unmodified gelatin, while the extension to break was lower.

Table 1. Tensile properties of crosslinked materials.

	Bovine gelatin	*Porcine gelatin*	*Bovine fibrinogen*
Ultimate Tensile Stress (kPa)	81	372	45
Extension to Break (%)	287	736	62

In vivo tissue sealing

We have previously shown that neither RuBTP nor SPS were cytotoxic at the concentrations routinely present after cross-linking, with SPS being very rapidly consumed during cross-linking, as determined using a PeroXOquant™ kit. The gelatin system was therefore examined for in vivo application in a model where high elasticity is needed.

Gelatin crosslinked *in situ* in a sheep lung incision model effectively sealed the wound from leakage of blood and air (Fig. 4). Gross morphological analysis at 2 weeks and 4 weeks after surgery showed no apparent inflammation at the wound site, and no signs of residual gelatin adhesive. Histology showed only minor inflammation at the sealed site 2 weeks after surgery, and no evidence of bleeding in the wound area following recovery.

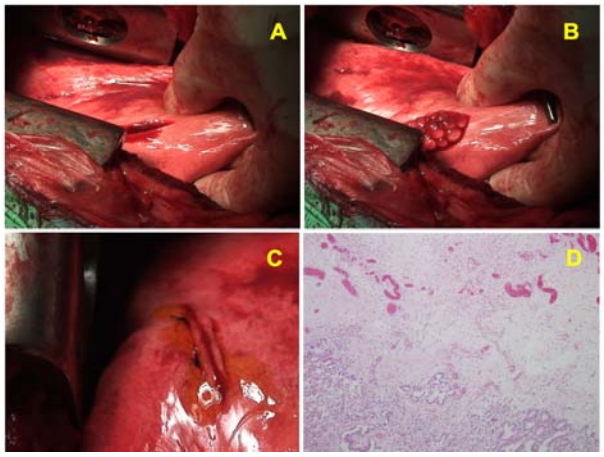

Fig. 4. Sealing of a lung incision using crosslinked gelatin; H&E staining of sealed wound site 2 weeks post surgery.

One aspect of this system is that photo-crosslinked gelatin can swell by ~250% within 24 h in PBS at 37°C. This swelling, which may not matter for certain applications, can be reduced using gelatin derivatised with Bolton-Hunter reagent. This phenolic-derivatised material shows negligible swelling, while retaining moderate elasticity.

Conclusion

We have described a new material produced by rapid, photochemical crosslinking of gelatin. The material has remarkable elasticity, strong tissue adhesive properties, is thermally stable and is compatible with cells and tissue *in vitro* and *in vivo*. The mechanical properties of the gelatin adhesive can be further manipulated, for example by modifying the Tyr content of gelatin, or by varying the type and concentration of components. It is suitable for various applications including clinical use and in composite materials that include particles, with gelatins being cheap, biodegradable and biocompatible.

References

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