

sprayed onto sheets as described in Example 2. The sheets are coarse fibered collagen hydrated by saline, which simulates a tissue surface. The total volume is approximately 0.5 ml. This formula gels in 18–15 sec. At 16 seconds, a second gel mixture of tetra-sulfhydryl PEG, di-maleimidyl PEG, both at 20% solids, and thrombin (700 NIH units/ml) of total gel mixture, total volume approx. 0.5 ml, are sprayed on top of the first gel. This second gel layer gels at about 2 minutes. At 3 min after the first gel is sprayed, 0.4 ml of re-calcified rabbit blood plasma, prepared as described above are layered on top of the PEG gel. This plasma clots 1.5 minutes after it is layered onto the PEG gel. The formation of a fibrin clot at this early time, compared to a non-thrombin control, is taken as evidence for active thrombin in the PEG gel.

#### Example 10

##### Gelation Using Powdered Formulations

10 mg of powdered tetra-SG PEG, 10,000 mol. wt., is spread on the surface of a piece of weighing paper. 10 mg of tetra-sulfhydryl PEG, 10,000 mol. wt., is dissolved in 50  $\mu$ l of P/C buffer. The sulfhydryl-PEG solution is loaded into a 1 cc syringe with a Haemedics (Malibu, Calif.) spray head and sprayed onto the SG-PEG on the weighing paper. The sprayed fluid is not stirred or mixed. It begins to gel in 27 seconds and forms a firm, rubbery layer by 2 min. This test shows that components in powdered form are also suitable for use in the present invention.

#### Example 11

##### Collagen-containing Compositions

Methylated collagen is prepared by the following process: bovine corium collagen is solubilized using pepsin and purified as described in U.S. Pat. No. 4,233,360. This purified, solubilized collagen is precipitated by neutralization into 0.2M sodium phosphate, pH 7.2. The precipitate is isolated by centrifugation to a final concentration of 70 mg/ml. The material is dried for two days, and then pulverized. Dry methanol containing HCl (to 0.1 N) is added (40 ml) and stirred for four days. Collagen is separated from the acidic methanol, vacuum dried and sterilized by irradiation. The final product is dissolved in water at a pH of 3–4.

For delivery as a sealant, 10 mg of the methylated collagen, 100 mg of tetra-functional sulfhydryl-PEG, 10,000 mol. wt., and 100 mg of tetra-functional SG PEG, 10,000 mol. wt., are dissolved in water at pH 3–4 to a final volume of 1 ml (first component). The second component is 1 ml of P/C Buffer. Each component is placed in a syringe and mixed and sprayed on the desired test site using a dual-syringe delivery system as described in Example 1. The applied mixture gels in less than 3 seconds.

The adhesive and cohesive properties of the gel are examined in a burst test. This test is conducted on a pressure gauge apparatus (PSI-Tronix, Model PG5000, Tulare, Calif.) connected by a pressure line to a circular sample plate with a 2 mm diameter central orifice. Sealant formulations are sprayed onto the plate to seal the orifice. To simulate bonding of the formulations to tissue, the sample plate has a circular sheet of coarse-fibered collagen fastened to it, with a 2 mm hole pierced into it and displaced 2–3 min from the sample plate orifice. Burst strength is measured as a function of the pressure it takes to force saline at a flow rate of 5 ml/min through the sealant gel.

The results are given below in Table 9.

TABLE 9

Burst Strength Measurements of Collagen-Containing Compositions	
Material	Burst Strength, mm Hg
Sulfhydryl-PEG/SG-PEG	100–180
Sulfhydryl-PEG/SG-PEG/Methylated Collagen	122–205

Both formulations have gel times less than 3 seconds. As shown above, the addition of collagen to the formulation enhances burst strength.

#### Example 12

##### Synthesis of “12-arm” PEG Compounds

A 12-arm electrophilic PEG compound is formed from 1 mole of 4-arm sulfhydryl PEG, 10,000 mol. wt., and 4 moles of 4-arm SG-PEG, 10,000 mol. wt. The resulting compound is depicted in FIG. 4a. As shown, the compound core is pentaerythritol PEG ether tetra-sulfhydryl and the end functional group is succinimide. As long as the functional groups are reactive with one another to form chemical bonds, the sulfhydryl group, X, can be replaced with other nucleophilic groups, such as NH<sub>2</sub>, etc., and the succinimidyl group, Y, can be replaced with other electrophilic groups, such as maleimide, carbonyl imidazole, or isocyanate. This method is also used to prepare the 12-arm nucleophilic PEG compound depicted in FIG. 4b by reacting 4 moles of 4-arm sulfhydryl PEG with 1 mole of 4-arm SG-PEG. The formation of these compounds from their respective 4-arm intermediates is also shown in FIG. 5. It should be understood that such reactions produce a heterogeneous population of activated PEG product, some having less than 12 arms, and some having more than 12 arms. As used herein, a “12-arm” PEG also refers to such heterogeneous reaction products that have an average of about 12 arms on each molecule.

##### 12a: 12, Arm Sulfhydryl PEG

Eight grams of pentaerythritol (polyethylene glycol)ether tetra sulfhydryl was dissolved in a mixture of 100 mL of methylene chloride and 100 mL of triethylamine. Two grams of pentaerythritol (polyethylene glycol)ether tetra succinimidyl glutarate in 40 mL of methylene chloride was slowly added with stirring at room temperature under argon overnight. The solvent was removed and the product was isolated by recrystallization in ethanol and dried.

##### 12b: 12 Arm Succinimidyl PEG

Two grams of pentaerythritol (polyethylene glycol)ether tetra succinimidyl glutarate was dissolved in 50 mL of methylene chloride. 0.5 grams of pentaerythritol (polyethylene glycol)ether tetra amine in 10 mL of methylene chloride was slowly added with stirring at room temperature under argon overnight. The solvent was removed and the product was isolated by recrystallization in ethanol and dried.

When the two compounds were tested for burst strength as described in Example 12, they demonstrated a burst strength of greater than 150 mm Hg and a gel time of less than 2 seconds.

We claim:

1. A method for preparing a biocompatible gel, comprising:
  - (a) forming a reactive composition by admixing a biocompatible crosslinking component A having m sulfhydryl groups with a biocompatible crosslinking com-