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Development of Bioelastic Material for Aspects of Wound Repair

Contract No. N00014-90-C-0265

TRIANNUAL PROGRESS REPORT FOR FIRST TRIMESTER OF YEAR 3

(September through December, 1992)

Bioelastics Research
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TRIANNUAL PROGRESS REPORT FOR FIRST TRIMESTER OF YEAR 3

(September through December, 1992)

A. PURPOSE OF THE CONTRACT:

Broad Goal: The synthesis and characterization of bioelastic materials designed for specific applications of wound healing.

Specific Goals: For materials with possible use as wound coverings, control of the temperature of transition by changes in hydrophobicity will be examined. In addition, the rate of water passage through the matrix will be determined. The research approach is the stepwise coordination of the synthesis and characterization of the materials. The first application phase was concerned primarily with synthesis of the basic polypentapeptide poly(VPGVG) and the analog with L-alanine substituted in position 3, poly(VPAVG) and mole fraction combinations thereof to achieve elastomeric matrices of varying elastic moduli. *And in the third year polypentapeptides containing chemical clocks are to be characterized for their rate of breakdown and their effects on drug release profiles.*

Peptide Syntheses: The three sets of syntheses are:

1) Synthesis of the basic polypentapeptides, poly(VPGVG) and poly(VPAVG) and mole fraction combinations thereof, poly[f_{A3}(VPAVG),f_{G3}(VPGVG)], and compounding of these polymers to fabrics such as gauze.

2) Synthesis of analogs of poly(VPAVG) with different hydrophobicities.

3) Synthesis of analogs with functional side chains to achieve chemical control of contraction under isothermal conditions.

4) *Synthesis of polymers containing chemical clocks to gain control of the rate of degradation.*

Physical Studies: The physical studies include:

1) Determination of the temperatures of transition.

2) Evaluation of the properties of aerosol sprays and foams on 37°C surfaces and cavities.

3) Determination of the rates of water loss through the synthetic elastic matrices or membranes (for those materials with potential for use as wound coverings or as vascular sleeves).

4) Mechanical studies including: stress strain, temperature dependence of length at constant force, temperature dependence of force at constant length and rates of contraction.

5) *Determination of the relative rates of degradation in relevant media.*

6) *The release of model drugs from cross-linked matrices and coacervates with and without chemical clocks*

B. SYNTHESES BEGUN IN THE FIRST TRIMESTER OF YEAR 03:

The synthesis of 10 grams of the following two polymers has begun:

1. poly[0.9(AVGVP),0.1(AFGVP)]
2. poly[0.8(AVGVP),0.2(AFGVP)]

Syntheses of these compounds have been undertaken to facilitate production of matrices of increased strength.

C. PROGRESS: IN USING BIOELASTIC MATERIALS AND THE ΔT_t MECHANISM IN DRUG DELIVERY

Living organisms develop and survive by achieving the capacity to convert available energy sources into motion and other functions required for life. This entails free energy transductions involving the intensive variables of mechanical force, temperature, pressure, chemical potential, electrochemical potential and electromagnetic radiation, e.g., visible and ultraviolet light. A common molecular mechanism whereby these energy conversions can occur is the ΔT_t-mechanism which utilizes inverse temperature transitions leading to increased order by hydrophobic folding and

assembly of protein on raising the temperature in water and by changing the temperature, T_t, at which the inverse temperature transition occurs using one of the above intensive variables to lower the value of T_t from above to below physiological temperature and thereby to drive hydrophobic folding and assembly.

The ΔT_t-Mechanism:[1,2] The ΔT_t-mechanism was discovered and developed using elastomeric polypeptides, a class of protein-based polymers called bioelastic materials; it is considered relevant to protein folding and function. The demonstrated and putative energy conversions, to date, are given in Figure 1. As represented in Figure 2A, poly(GVGVP) is extended at low temperature and hydrophobically folds on raising the temperature with the capacity to lift a weight and perform work. This is thermomechanical transduction. Now if the transition occurs as in curve b and an intensive variable is introduced which lowers the transition as in curve a, then complete contraction is achieved when the temperature is within the range indicated by c. This is the ΔT_t-mechanism. Means whereby the value of T_t can be changed are shown in Figure 2B, *ii* through *vi*. Figure 2C depicts the elastic model protein bands achieved by 20 Mrad g-irradiation cross-linking of poly(GVGVP), or a suitable modification undergoing contraction (a) or relaxation (b) by the means indicated in Figure 2D. Contraction/relaxation can be a de-swelling/swelling process with a volume change in water of a factor of 10.

Biocompatibility of Bioelastic Materials: [3] Employing eleven recommended biological tests for determining biocompatibility, the basic materials—poly(Gly-Val-Gly-Val-Pro) and its 20 Mrad γ-irradiation cross-linked matrix, X^{20}-poly(GVGVP)—have been demonstrated to exhibit remarkable biocompatibility.[3] Accordingly, these materials become candidates for effecting controlled release of pharmaceuticals. In this regard, it is significant, when implanted in the rat and the rabbit for periods of up to several months, that there is no fibrous capsule formation around X^{20}-poly(GVGVP).[4,5] This allows that the matrix itself can control the rate of release without the limitations imposed by an encapsulating fibrous protein coat. After months of being implanted within the peritoneal cavity or under the conjunctiva of the eye, X^{20}-poly(GVGVP) remains a soft, transparent, elastic matrix which retains its capacity to exhibit transductional behavior.

The contracted transparent elastic matrix, X^{20}-Poly(GVGVP), in spite of its forming by means of hydrophobic folding and assembly, is 50% water by weight under physiological conditions;[6-8] it can be loaded with agents by various means, e.g., most simply by swelling in the presence of the agent dissolved in water and then by contracting to expel excess water. X^{20}-poly(GVGVP), for example, can increase in volume some 10-fold on lowering the temperature from 37°C to 20°C. This swell-doping is particularly effective for agents with relatively hydrophobic moieties and limited solubility in water such as biebrich scarlet and methylene blue. These properties also suggest useful loading with pharmaceuticals such as steroids, peptides (e.g., enkephalin), oligonucleotides and even small proteins.

Controlled Release. Diffusional release is a mechanism available to essentially all drug-laden matrices. Degradational release of agents is possible with a more limited subset of matrices including those comprised of polypeptides and polyesters. The bioelastic polymers can also be synthesized with periodic esters in the backbone but also can be designed to contain sites for proteolytic enzymes anticipated in the milieu of the implant site or even for proteolytic enzymes doped within the matrix itself.

Perhaps most significantly, the present materials introduce a versatile set of transductional release processes where there becomes the potential for increased control of release. For example, there can be introduced chemical clocks such as the carboxamides of asparagine or glutamine residues wherein, depending on the sequence in which the residue occurs, the carboxamide will hydrolytically cleave at the matrix-milieu interface with a particular half-life.[9] With a properly designed matrix this can result in a raising of T_t above physiological temperature with the consequences of dramatic swelling, enhanced diffusional release and enhanced degradation.[10] Introduction of an occasional ester in the backbone by replacing a glycine residue with a glycolic acid residue allows for chain breakage at the matrix interface, and also results in formation of carboxylate which would enhance swelling. Changes in any of the intensive variables of temperature, pressure, chemical potential, electrochemical potential and electromagnetic radiation could be used to control the rate of release by swelling and degradation or conversely to control the rate of release by driving contraction of an envelope to expel contents.[1,2]

Model Elastic Protein-based Polymers for Controlled Release: As an initial effort at introducing transductional capacities into controlled release, a series of sequential polypeptides have been prepared. These are: I, poly(GVGVP); II, poly[0.85(GVGVP), 0.15(GVG$_C$VP)]; III, poly[0.90(VPGVG), 0.10(VPG$_C$VG)]; and IV, poly[0.94(VPGVG), 0.06(VPG$_C$NG)] where V = Val, P = Pro, G = Gly, G$_C$ = glycolic acid and N = Asn(asparagine). The rates of breakdown were followed using the time dependence of the temperature, T_t, of the inverse temperature transition when in 0.2 N phosphate to maintain pH in the 7.0 to 7.5 range. Using the 8-day time interval from 6 to 14 days which is quite linear for all polymers, the ΔT_t/8 days was 0 for I, 2.7°C for II, 1.6°C for III and 3.2°C for IV. As these polymers contained different mole fractions for the pentamer having the chemical clock, the values were normalized for that variable. This gave 0°, 18°, 16° and 53°C, respectively. Accordingly, there appeared to be little difference in the rate of breakdown of the glycolic ester whether in VG$_C$V or the PG$_C$V sequence. However, the combination of a carboxamide in the side chain and an ester in the backbone increased the rate for swelling and breakdown some three-fold.

Further, beginning efforts at controlled release have involved swell-doping of X^{20}-poly(GVGVP) matrices in the form of thin discs with biebrich scarlet and methylene blue. Both of these agents are taken very well into the matrix, and, after initial rinsing and an initial burst, there is a period approaching a week where release is sustained in the 50 nanomole to 15 nanomole/day range.

EXPERIMENTAL

Polypentapeptide Syntheses

The syntheses of VPGVG and GVGVP have been previously described.[11,12] For conformational reasons, that is, due to the Pro^2-Gly^3 Type II β-turn, the usual numbering of the pentamer is Val^1-Pro^2-Gly^3-Val^4-Gly^5.[1,12] The pentamers containing glycolic acid (G_C) and asparagine (N) were synthesized as VPG_CVG, VPG_CNG and GVG_CVP by the solution phase method. It has been found in our previous studies that the highest molecular weights were obtained when the pentamer permutation with Pro at the carboxyl terminus (GVGVP) and para-nitrophenol (ONp) were used for carboxyl activation.[12] Here, however, the VPGVG permutation was used with Gly at the C terminus for pentamers substituted in position 3 with the glycolic acid residue and with 1-ethyl-3-dimethylaminopropylcarbodiimide (EDCI) as the polymerizing agent. The reasons for these changes were (1) with GVGVP permutation, glycolic acid at position 3 occurs at the N terminus which may be difficult to polymerize under normal conditions necessitating in these cases the use of the VPGVG permutations, and (2) at the end of the polymerization, the terminus ONp moieties were removed by base treatment with 1 N NaOH which would hydrolyze ester bonds present in the polymers. Moreover, our previous studies[3,13] indicate that using EDCI with Pro at the carboxyl terminus also gives equally good polymers which were identical in transition temperature, carbon-13 nuclear magnetic resonance (NMR) spectra, amino acid analyses and biological studies. When the VPGVG permutation has been used with ONp, the temperature, T_t, of the inverse temperature transition has been higher.

The peptides Boc-Val-Pro-OBzl, Boc-Val-Gly-OBzl, Boc-Val-Pro-Gly-Val-Gly-OH and Boc-Gly-Val-Gly-Val-Pro-OH were prepared as previously described.[11,12] Pentamer purity prior to polymerization is a critical factor in obtaining high molecular weight polymers in good yield as impurities can result in termination of the polymerization process. The protected peptides were characterized by carbon-13 nuclear magnetic resonance before polymerization to verify the structure and purity. Thin layer chromatography (t. l. c.) was performed on silica gel plates obtained from Whatman, Inc., with the following solvent systems: R_f^1, $CHCl_3$:CH_3OH:CH_3COOH (95:5:3); R_f^2, $CHCl_3$:CH_3OH:CH_3COOH (90:10:3); R_f^3, $CHCl_3$:CH_3OH:CH_3COOH (85:15:3). The compounds on t. l. c. plates were detected by UV light, by spraying with ninhydrin, or by chlorine/tolidine spray. All Boc amino acids, N,N-diisopropylcarbodiimide and HOBt were purchased from Advanced Chem. Tech. (Louisville, Kentucky). EDCI was obtained from Bachem, Inc. (Torrance, California). Glycolic acid and carbonyldiimidazole were purchased from Aldrich Chemical Company(Milwaukee, WI).

N,N-Diisopropyl-O-benzylisourea. Benzyl alcohol (42.7 g, 0.4 mol) was added with stirring to a mixture of cuprous chloride (0.15 g) in N,N-diisopropylcarbodiimide (50.0 g, 0.4 mol) over a period of 30 min at 0°C. After an additional 1 hr at 0°C, the reaction was stirred at room temperature for 18 hr to ensure complete reaction. The volume was then doubled with hexane and the solution was applied to a filter pad of neutral alumina to remove copper salts. The product was eluted

with a total volume of 1 L of hexane, and the solvent was evaporated under reduced pressure and dried.

Benzyl-Glycolic acid (1). Glycolic acid (7.61 g, 0.1 mol) was added to N,N-diisopropylbenzylisourea (23.3 g, 0.1 mol) with stirring. The mixture became very viscous within 10 min and was stirred intermittently for 1 hr. The volume was then increased to 200 mL with THF, and the mixture was stirred for 48 hr at room temperature. After cooling the mixture to -15°C, the diisopropylurea was removed by filtration and the THF was evaporated under reduced pressure to give 15.26 g (yield, 91.8%) of benzyl glycolate.

Boc-Val-Pro-Glc-OBzl (2). A solution of Boc-Val-Pro-OH (9.43 g, 0.03 mol) in dry methylene chloride (50 mL) was cooled to 0°C with stirring and treated with a solution of carbonyldiimidazole (4.86 g, 0.03 mol) in methylene chloride (50 mL) over 30 min. After stirring for an additional 20 min at 0°C, benzyl glycolate (5.0 g, 0.03 mol) was added over a 30 min period. The reaction mixture was maintained at 0°C for a further 2 hrs and then at room temperature for 3 days. The mixture was evaporated to a thick oil which was dissolved in chloroform and extracted with water, 20% citric acid, water, saturated sodium bicarbonate, water and dried over sodium sulfate. The solvent was removed under reduced pressure and the resulting oil was recrystalized from ether/petroleum ether to obtain 9.0 g (yield, 64.84%) of **2**: R_f^1, 0.48; R_f^2, 0.59.

Boc-Asn-Gly-OBzl (3). A mixture of Boc-Asn-ONp (3.53 g, 0.01 mol), Gly-OBzl.p-Tosylate (3.77 g, 0.01 mol) and HOBt (1.35 g, 0.01 mol) in DMF (50 mL) was stirred at room temperature for 2 days maintaining the pH at 7.5 to 8.0 with NMM. The solvent was removed under reduced pressure and worked up by acid-base extraction. The resulting gum was recrystalized from ether to obtain 2.8 g (yield, 73.68%) of **3** : R_f^1, 0.26; R_f^2, 0.35.

Boc-Val-Pro-Glc-Val-Gly-OBzl (4). Compound **2** (9.0 g, 0.0195 mol) was dissolved in dry THF (100 mL), and 10% palladium on activated charcoal(1.0 g) was added. This mixture was hydrogenated at 50 psi for 6 hr and the catalyst was removed by filtration through celite. The filtrate was evaporated *in vacuo* and dried to give the acid, Boc-Val-Pro-Glc-OH.
Boc-Val-Gly-OBzl (3.3 g, 0.009 mol) was deblocked by stirring for 1.5 hr in 4 N HCl in dioxane. Excess HCl and dioxane were removed under reduced pressure, triturated with ether, filtered, washed with ether and dried.

A solution of Boc-Val-Pro-Glc-OH (3.35 g, 0.009 mol) and HOBt (1.35 g, 0.01 mol) in DMF was cooled to -15°C with stirring and EDCI (1.97 g, 0.01 mol) was added. After 20 min a pre-cooled solution of the above hydrochloride salt and NMM (0.99 mL, 0.01 mol) was added and the reaction mixture stirred overnight at room temperature. The mixture was evaporated to a thick oil which was dissolved in chloroform. This solution was extracted with water, 10% citric acid, water, 5% sodium bicarbonate, water and dried over sodium sulfate. The solvent was removed under reduced pressure and the resulting oil was dissolved in ether and precipitated from petroleum ether.

The solid was filtered, washed with petroleum ether and dried to obtain 4.1 g (yield, 73.61%)of **4**: R_f^2, 0.51; R_f^3, 0.64.

Boc-Val-Pro-Glc-Asn-Gly-OBzl (5). Compound **3** (2.9 g, 0.0075 mol) was deblocked with trifluoroacetic acid and coupled to Boc-Val-Pro-Glc-OH (2.75 g, 0.0074 mol) using EDCI with HOBt in the same manner as that described for **4** to give 2.82 g (yield, 60.1%) of **5**: R_f^2, 0.33; R_f^3, 0.41.

Boc-Val-Glc-OBzl (6). Compound **1** (7.5 g, 0.04 mol) was coupled to Boc-Val-OH (9.6 g, 0.04 mol) using carbonyldiimidazole (7.3 g, 0.045 mol) following the same procedure described for the preparation of **2** to give 12.5 g (yield, 76.05%) of **6**: R_f^1, 0.55; R_f^2, 0.65.

Boc-Gly-Val-Glc-OBzl (7). Compound **3** (8.04 g, 0.022 mol) was deblocked using HCl/dioxane and coupled to Boc-Gly-OH (3.5 g, 0.02 mol) using EDCI with HOBt as described in the preparation of **4** to obtain 7.5 g (yield, 88.7%) of **7**: R_f^1, 0.44; R_f^2, 0.52.

Boc-Gly-Val-Glc-Val-Pro-OBzl (8). Compound **7** (4.65 g, 0.011 mol) was hydrogenated into free acid then coupled to HCl. VP-OBzl (obtained by deblocking 4.45 g of Boc-Val-Pro-OBzl with HCl/dioxane) using EDCI in the presence of HOBt and following the same procedure as described for **4** to obtain 6.0 g (yield, 91.4%) of **8:** R_f^1, 0.21; R_f^2, 0.29.

Poly(Gly-Val-Gly-Val-Pro) (I). Boc-Gly-Val-Gly-Val-Pro-OH (2.11 g, 0.004 mol) was deblocked with TFA, and a one-molar solution of TFA salt in DMSO was polymerized for 12 days using EDCI (2 equiv.) as the polymerizing agent with HOBt (1 equiv.) and 1.6 equiv. of NMM as base. The polymer was dissolved in water, dialyzed using 3500 mol wt cut-off dialysis tubing for one week and lyophilized . It was then dialyzed using 50kD mol wt cut-off dialysis tubing for one week and lyophilized to obtain 1.09 g (yield, 66.84%) of **I.**

Poly[0.9(Gly-Val-Gly-Val-Pro), 0.1(Gly-Val-Glc-Val-Pro)] (II). Compound **8** (6.0 g, 0.0096 mol) was dissolved in THF (60 mL), and 10% palladium on charcoal (0.6 g) was added. This mixture was hydrogenated at 40 psi for 6 hrs; then the resulting residue was triturated with ether, filtered and dried to obtain the acid.

Boc-Gly-Val-Gly-Val-Pro-OH (10.55 g, 0.02 mol) and Boc-Gly-Val-Glc-Val-Pro-OH (1.18 g, 0.0022 mol) were deblocked together with TFA and polymerized using the same procedure for the compound **I** to obtain 4.3 g (yield, 47.3%) of **II.**

Poly[0.9(Val-Pro-Gly-Val-Gly), 0.1(Val-Pro-Glc-Val-Gly)] (III). Compound **4** (4.1 g, 0.0066 mol) was hydrogenated as described above to obtain 3.1 g (yield, 88.57%) of the acid. This acid (1.32 g, 0.0025 mol) was mixed with Boc-Val-Pro-Gly-Val-Gly-OH (11.87 g, 0.022 mol) and deblocked together with TFA. The polymerization, dialysis and lyophilization were carried out using the same procedure described for the polymer **I** to obtain 4.1 g (yield, 40.04%) of **II.**

Poly[0.9(Val-Pro-Gly-Val-Gly), 0.1(Val-Pro-Glc-Asn-Gly)] (IV). Compound **5** (2.8 g, 0.0044 mol) was debenzylated following the same procedure described above to obtain 1.8 g (yield, 75%) of the acid. This acid (1.35 g, 0.0025 mol) and Boc-Val-Pro-Gly-Val-Gly-OH (11.87 g, 0.022 mol) were deblocked, polymerized, dialysed and lyophilized using the same procedure described for the polymer **I** to obtain 3.8 g (yield, 37.3%) of **IV**.

The purity and the composition of the final products was checked by carbon-13 nuclear magnetic resonance and amino acid analyses. Based on the amino acid analyses, the more correct statement of the formulae for the polymers with mixed pentamers would be **II**: poly[0.85(GVGVP), 0.15(GVG_CVP)], **III**: poly[0.9(VPGVG), 0.1(VPG_CVG)] and **IV**: poly[0.94(VPGVG), 0.06(VPG_CNG)].

ABBREVIATIONS: Boc, tert-butyloxycarbonyl; ONp, para-nitrophenol; OBzl, benzyl; EDCI, 1-ethyl-3-dimethylaminopropyl carbodiimide; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; NMM, N-methylmorpholine; DMSO, dimethylsulfoxide; DMF, dimethylformamide; THF, tetrahydrofuran; V (Val), valine; P (Pro), proline; G (Gly), glycine; N (Asn), asparagine; G_C (Glc), glycolic acid.

Determination of T_t: Below a certain temperature each of the polymers I through IV is soluble in water. On raising the temperature, there is the onset of aggregation resulting in a phase transition to form a more-dense, polymer-rich, viscoelastic state called a coacervate. The onset of the transition can be followed spectrophotometrically by the temperature profile of turbidity formation where the temperature for half-maximal turbidity is designated as T_t, the temperature of this inverse temperature transition in which increased hydrophobicity lowers the value of T_t and increased polarity as the formation of carboxylates markedly increases T_t.

For these elastic protein-based polymers, it has been found that 40 mg/mL is the high concentration limit above which increasing concentration no further lowers the value of T_t. Accordingly, all polymers were studied beginning with concentrations of 40 mg/mL. The polymers were dissolved in 0.2 N phosphate at pH 7.5 in order to maintain a near constant pH during the breakdown of carboxamides and esters to polar carboxylates. The value of T_t for each polymer was determined at zero time and then the samples were incubated at 37°C in a rocker device for 24 hrs and the temperature lowered to achieve dissolution and a new determination of T_t. This process was repeated each 24 hours for 21 days. Increases in the value of T_t with time at 37°C was due to hydrolysis of carboxamides and esters. The temperature profiles of turbidity formation were run on a Pye-Unicam 8610 spectrophotometer at 400 nm.

Formation of the X^{20}-poly(GVGVP) Bioelastic Matrix: The coacervate state of poly(GVGVP) was placed in a mold capable of forming sheets 0.35 mm in thickness and cross-linked at the Auburn University Nuclear Science Center with a 20 Mrad dose of γ-irradiation. Discs of

diameter 5.45 mm and a thickness of 0.35 mm were punched from the bioelastic sheet equilibrated in water at 37°C.

Loading the Discs with Biebrich Scarlet and Methylene Blue: A 47 mL, 0.1M solution of biebrich scarlet and of methylene blue were each added to a disc of volume 8.2 mL. As the 37°C state of X^{20}-poly(GVGVP) can expand on lowering the temperature in water to take up a 10-fold volume of water, the 5 to 6-fold volume was entirely taken into the disc and the swollen disc was allowed to stand overnight at 4°C. The temperature was then raised to 37°C causing the extrusion of water and excess biebrich scarlet. Of the 4.7 mmoles of biebrich scarlet in the first experiment, 2.5 mmoles were retained within the 1 volume of contracted disc and 2.2 mmoles were released to the 5.7 volumes of extruded water and, in the second experiment, the values were 1.7 and 3.0 mmoles, respectively. Thus, the swell-doping occurred with a several-fold greater partitioning into the disc of X^{20}-poly(GVGVP). In the first experiment with a longer time at 4°C, 52% of the biebrich scarlet was retained by the contracting disc and in the second experiment with a shorter time at 4°C, 36% of the biebrich scarlet was retained by the disc. An extinction coefficient for biebrich scarlet of 1.77×10^4 liters/mole-cm at 505 nm was used.

After three brief rinsings at 45°C with 0.5 mL each of phosphate buffered saline (PBS), 0.15 N NaCl and 0.01 M phosphate, at pH 7.4, the 24 hr release into a 1 mL aliquot of PBS at 37°C was followed daily for eleven days. The release data were obtained using both the Pye-Unicam 8610 and the AVIV 14 DS spectrophotometers.

RESULTS

Effect of Chemical Clocks on T_t, the Temperature of the Inverse Temperature Transition: The data of Figure 3 demonstrate the concept and potential utilization of chemical clocks. Polymer I, poly(GVGVP), exhibits a constant value for T_t over the three-week period. This polymer is stable under the conditions of 0.2 M phosphate, pH 7.0 to 7.5. Polymers II and III, poly[0.85(GVGVP),0.15(GVGcVP)] and poly[0.90(VPGVG),0.10(VPG$_c$VG)], contain the glycolic acid residue (G_c) and are capable of hydrolytic cleavage with rupturing of a backbone bond and production of a carboxylate. Both the decrease in chain length and the formation of the carboxylate increase the value of T_t. The value of T_t for these polymers appears to change at different rates in the 6 to 14-day range. The slopes are 0.332°C/day for polymer II and 0.223°C/day for polymer III. When correction is made for the mole fraction differences of the G_c-containing pentamers, however, that is 0.332°C/0.15 mole fraction pentamer-day and 0.223°C/0.1 mole fraction pentamer-day, the values are seen to be identical, 2.2°C/pentamer-day. Therefore, there appears to be no difference in stability for G_c in the VG_cV sequence or in the PG_cV sequence. The slope for the same period of time, 6 to 14 days, for polymer IV, poly[0.94(VPGVG),0.06(VPG$_c$NG)] is 0.39°C/day which when corrected for mole fraction, i.e., 0.39/0.06, becomes 6.5°C/pentamer-day. Thus, the combination of both G_c and N results in a three-fold greater slope per pentamer. As will be discussed below, the critical issue becomes the time required for T_t to reach the operating temperature.

Diffusional Release of Biebrich Scarlet from X^{20}-poly(GVGVP): As shown in Figure 3 the value of T_t for polymer I, poly(GVGVP) does not change in phosphate buffer. Accordingly release from X^{20}-poly(GVGVP) may be considered to be entirely diffusional with neither degradation nor change in the degree of swelling altering the release rate.

As noted in the Methods section, it is possible to load the matrix, X^{20}-poly(GVGVP) in the form of a thin disc, with biebrich scarlet simply by lowering the temperature in the pressure of a 5 to 6-fold volume of a 0.1 M solution. In Figure 4 the release of the loaded contracted matrix at 37°C is seen to occur over a period of more than ten days.

Initial release rates are of the order of several hundred nanomoles per day. In the period of 5 to 11 days release rates go from 50 nanomoles/day to 15 nanomoles/day. Thus, even with this thin disc a reasonably narrow range of release rates can be sustained for much of a week.
Some differences have been observed depending on the initial equilibration time for swell-doping at low temperature. While variations in the initial washes may be responsible in part, the sample having had the longer equilibration time initially exhibited lower release rates but then higher release rates after five days.

Qualitatively similar data have been obtained using methylene blue as the drug release model, but the study is complicated by an aggregation dependent absorption in the concentration range of interest in the study.

DISCUSSION

It is seen by Figure 4 that the property of an inverse temperature transition can be used to load an elastomeric polypeptide matrix with drug. This concept of lowering the temperature to achieve swell-doping and of contracting to expel excess water while retaining drug is clearly demonstrated. In the case of biebrich scarlet this loading process has achieved a concentration of 0.3 M in the contracted matrix or approximately 300 mmoles in one cm^3 (one mL) of volume. This is approximately one biebrich scarlet molecule for three pentamers or one per turn of the β-spiral. It is apparent that the X^{20}-poly(GVGVP) matrix itself can function as a substantial reservoir for drugs having the chemical composition of biebrich scarlet.

The most limiting case for testing the potential of X^{20}-poly(GVGVP) to achieve a sustained release is to use a thin monolith. In the present report a volume of 8.2 mL was used within the shape of a thin (0.035 cm thick) disc. Given this challenge, it is encouraging to see the release profile of Figure 4. It is not unreasonable to anticipate with thicker monoliths that a desired release range, controlled by concentration of the loaded matrix, could be sustained for periods of weeks. A visual sense of the thinness of the present disc is seen in Figure 5 where a stack of 122 discs are required to produce a volume of one mL.

With the introduction of the capacity of the matrix to degrade as would be the case for polymers II and III, the release ranges could be made narrower and to extend over longer periods of time. With the introduction of transductional release, using any of the free energy inputs of Figures 1 and 2 or simply using chemical clocks such as carboxamides breaking down to carboxylates at the matrix-milieu interface to control the rate of matrix surface swelling, one can expect to gain even finer control of the quantity of the drug to be released and to do so over even longer periods of time. Variations in the composition of the matrix, made possible with knowledge of the hydrophobicity scale[14], can be used to change affinities for different drugs or to change the affinity for a given drug. One could also conceive of constructs which could give rise to oscillatory release over substantial periods of time.

Use of the Rate of Change of T_t to Effect Transductional Release by Swelling: As long as the value of T_t is 15°C below the physiological temperature, release of drug would be limited to diffusion from the contracted matrix, but as T_t increased from 15°C below 37°C to physiological temperature the affected surface layer of matrix will undergo a swelling to about ten times its original volume, thereby facilitating diffusional release. This transductional change would not only enhance the release rate but would result in a matrix more susceptible to proteolytic degradation and should the chemical clock include the glycolic acid residues there would be the simultaneous hydrolytic cleavage to cause chain rupture. It may be noted that, in the absence of such transductional effects, the contracted matrix, X^{20}-poly(GVGVP), has appeared to remain intact and transparent for many months in the peritoneal cavity of rats[5] and for two months beneath the conjunctiva of the eye in rabbits[4].

If the desired therapeutic dose for local release of biebrich scarlet to stimulate wound healing were in the range of 15 to 50 mmoles/day to be maintained for a week, then the swell-doped disc of the present study, pretreated by a four-day washout, would suffice. If higher doses were sought over a longer period, then a larger monolith would be used. An example of a particular monolith of a height equivalent to a stack of 122 discs of the present study is shown in Figure 5. Should the introduced chemical clock be such that the equivalent of one disc were removed every 2.5 days, then the monolith could deliver a mean dose as high as one mmole/day for a period of 300 days.

SUMMARY

Bioelastic matrices are capable of effecting drug release by means of diffusion, degradation, transduction or a designed combination of any of the three. With proper design the transductional release can utilize free energy inputs including changes in the intensive variables of mechanical force, temperature, pressure, chemical potential, electrochemical potential and electromagnetic radiation. In the present report the feasibility of chemical clocks to introduce both degradational and transductional release is demonstrated and diffusional release from a limiting case of a thin disc of the simplest biocompatible matrix is demonstrated using the drug biebrich scarlet which has wound repair efficacy. It is seen for example that a local release of 50 to 15 nanomoles/day of biebrich scarlet could be sustained for much of a week. This biocompatible matrix, X^{20}-poly(GVGVP), has been found

not to become coated with a fibrous capsule even after months of implantation within the peritoneal cavity and under the conjunctiva of the eye. It is suggested that larger constructs utilizing chemical clocks could result in sustained release of the order of 1 mmole per day for much of a year.

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D. PUBLICATIONS SINCE LAST REPORT (Copies Attached):

1. D. W. Urry, D. C. Gowda, C. Harris, R. D. Harris and B. A. Cox, "Development of Bioelastic Materials as Biocompatible, Transducible and Degradable Drug Delivery Matrices," Polym. Preprints, Div. Polym. Chem., Am. Chem. Soc., **33** (2), 84-85, 1992.
2. D. W. Urry, A. Nicol, D. C. Gowda, L. D. Hoban, A. McKee, T. Williams, D. B. Olsen and B. A. Cox, "Medical Applications of Bioelastic Materials," In Biotechnological Polymers: Medical, Pharmaceutical and Industrial Applications. A Conferences in Print (Charles G. Gebelein, Ed.), Technomic Publishing Co., Inc., Atlanta, Georgia, 1993 (in press).
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Demonstrated and Putative Energy Conversions Using Molecular Machines of the T_t–type

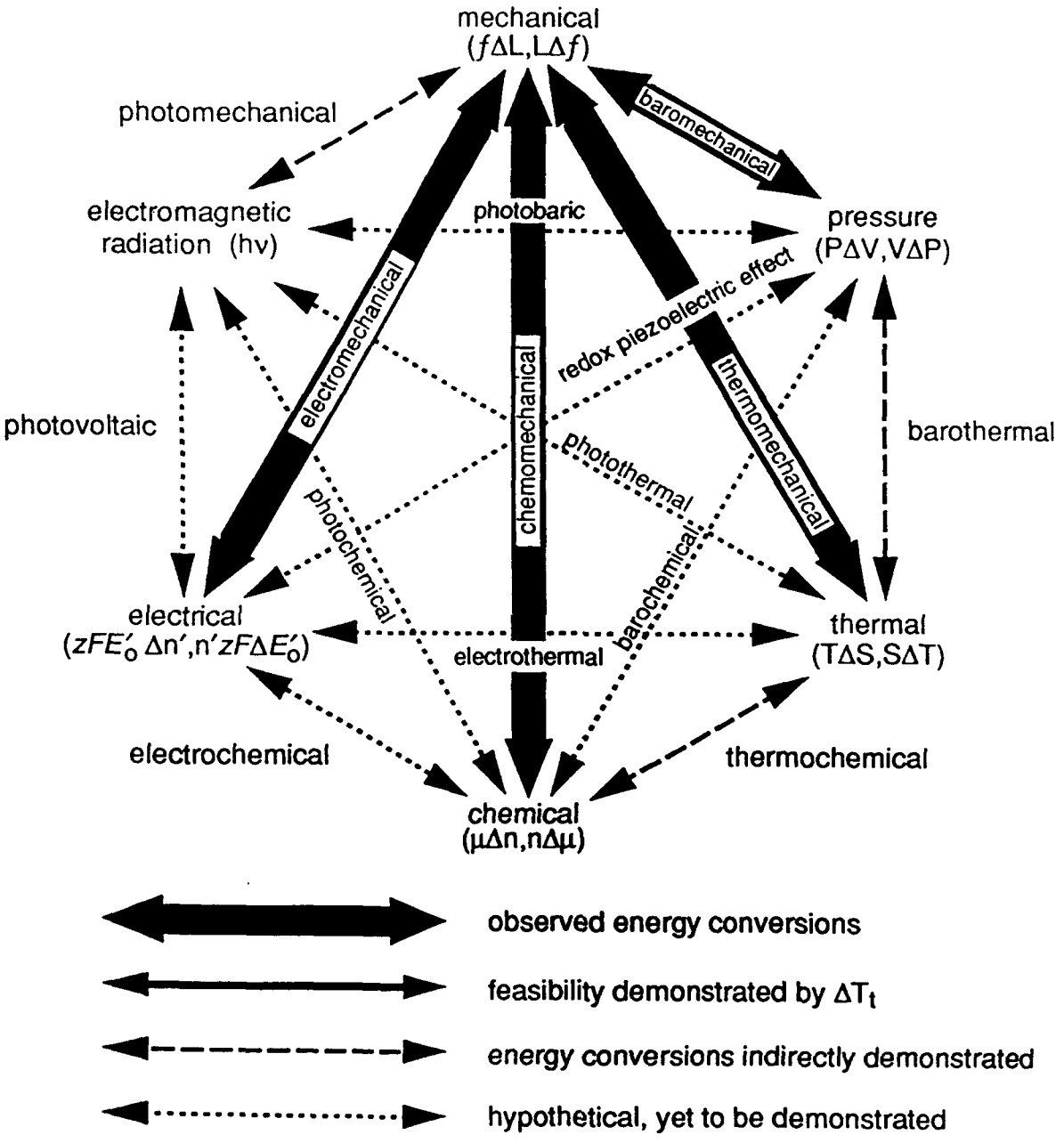
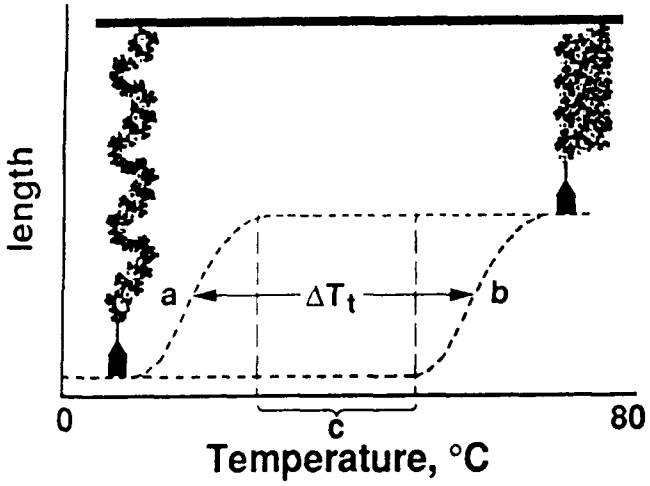


FIGURE 1

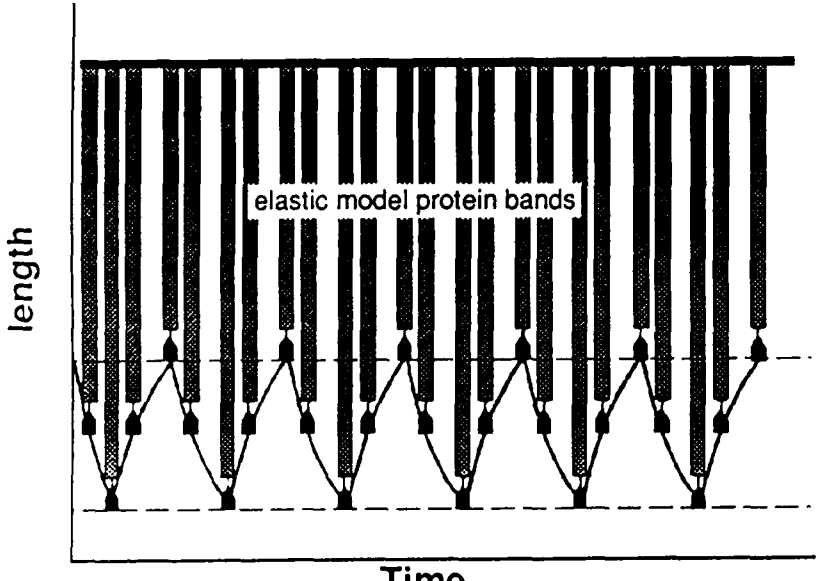
Free Energy Transductions by the ΔT_t – Mechanism

A. Thermally-driven Contraction (Thermomechanical Transduction)



	a. Means of driving contraction	b. Means of effecting relaxation
	i. raising the temperature	*i.* lowering the temperature
ΔT_t	*ii.* intrinsic chemical change e.g. $COO^- \longrightarrow COOH$ (adding acid)	*ii.* intrinsic chemical change e.g. $COOH \longrightarrow COO^-$
	iii. extrinsic chemical change e.g. adding salt	*iii.* extrinsic chemical change e.g. wash out of salt
	iv. reducing prosthetic group e.g. reduction of nicotinamide	*iv.* oxidizing prosthetic group
	v. release of pressure when aromatic residues present	*v.* application of pressure when aromatic residues present
	vi. dark reversibility of light reaction**	*vi.* light-effected photochemical reaction of suitable chromophore**

B. Means of Driving Contraction/Relaxation



Isothermal Energy Conversions When in the Temperature Range c

	i. thermomechanical	*ii.* chemomechanical (intrinsic)	*iii.* chemomechanical (extrinsic)	*iv.* electromechanical*	*v.* baromechanical*	*vi.* photomechanical**
a.	40° C	pH 2	+ salt	red.	– P	dark
b.	20° C	pH 7	– salt	ox.	+ P	light

[*] reversibility has yet to be demonstrated [**] The ΔT_t has yet to be unambiguously demonstrated

FIGURE 2

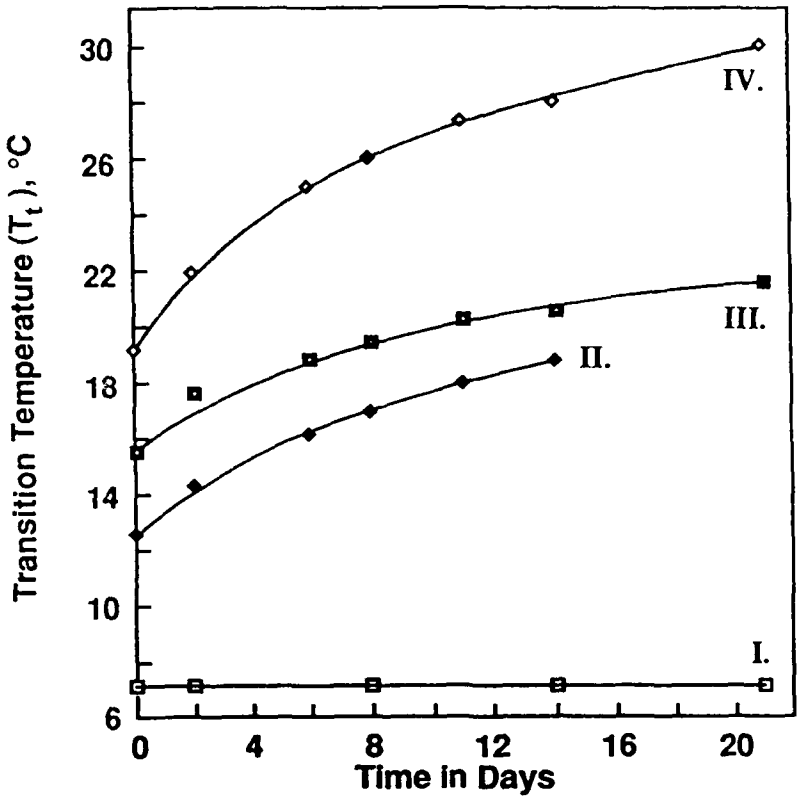

Transition Temperature (T_t), °C
30
26
22
18
14
10
6
0
4
8
12
16
20
Time in Days
IV.
III.
II.
I.

FIGURE 3

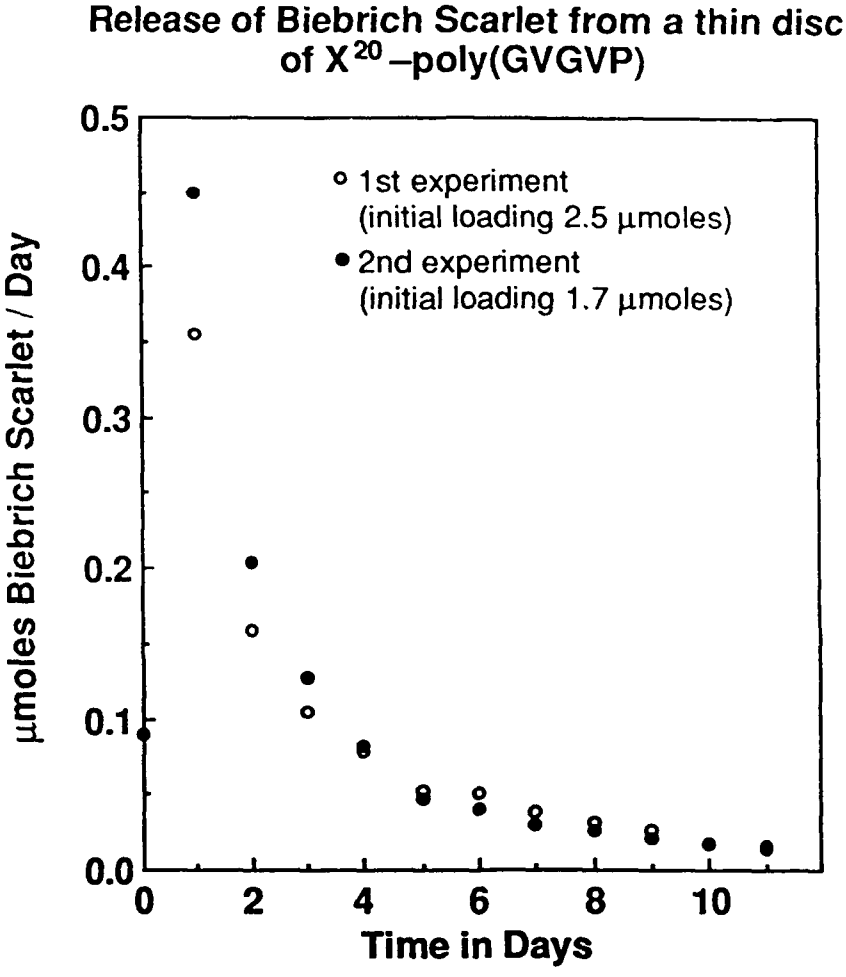

Release of Biebrich Scarlet from a thin disc of X20–poly(GVGVP)
µmoles Biebrich Scarlet / Day
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
Time in Days
1st experiment
(initial loading 2.5 µmoles)
2nd experiment
(initial loading 1.7 µmoles)

FIGURE 4

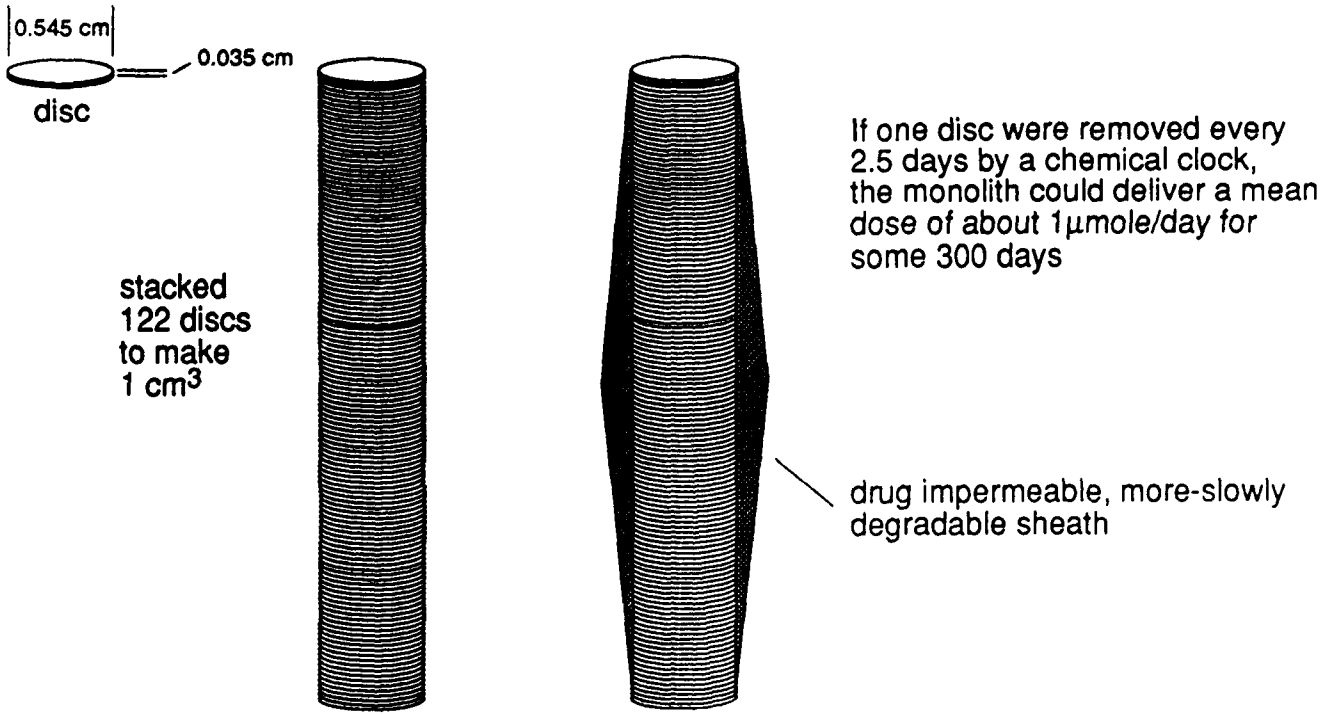

0.545 cm
0.035 cm
disc
stacked
122 discs
to make
1 cm3
If one disc were removed every
2.5 days by a chemical clock,
the monolith could deliver a mean
dose of about 1μmole/day for
some 300 days
drug impermeable, more-slowly
degradable sheath

FIGURE 5

#399

DEVELOPMENT OF BIOELASTIC MATERIALS AS BIOCOMPATIBLE, TRANSDUCIBLE AND DEGRADABLE DRUG DELIVERY MATRICES

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INTRODUCTION

The polypentapeptide, poly(Val[1]-Pro[2]-Gly[3]-Val[4]-Gly[5]), or poly(VPGVG), and its γ-irradiation cross-linked matrix are biocompatible as shown by a series of eleven tests performed by North American Science Associates; those tests are: [1] mutagenicity, [2] cytoxicity, [3] systemic toxicity, [4] intracutaneous toxicity, [5] muscle implantation, [6] acute intraperitoneal toxicity, [7] systemic antigenicity, [8] dermal sensitization, [9] pyrogenicity, [10] blood clotting, and [11] hemolysis. Reports of these studies are in archival files at North American Science Associates, Inc. (NAmSA), 2261 Tracy Road, Northwood, Ohio 43619, and they have been reviewed elsewhere (1).

Poly(VPGVG) undergoes a modulatable inverse temperature transition in which it is soluble (miscible) with water in all proportions at temperatures below 20°C but on raising the temperature to 40°C poly(VPGVG) undergoes a hydrophobic folding and assembly transition leading to the formation of a viscoelastic (coacervate) phase which is about 50% water, 50% peptide by weight (2). When the coacervate phase, formed at 40°C, is γ-irradiation cross-linked by a 20 x 10^6 radiation absorbed dose (20 Mrad), it forms an elastomeric matrix which swells on lowering the temperature to result in a tenfold increase in volume (3). Thus the cross-linked poly(VPGVG) forms a hydrogel-like material that would be suitable for drug delivery. Of added interest is that the breakdown products are zwitterionic amino acids in which there is no acid release as occurs with the hydrolysis of esters, for example.

What makes this new class of compliant biomaterials (bioelastic materials) of particular interest is the capacity to have the temperature, T_t, of the inverse temperature transition varied as the result of a change in any of a number of intensive and extensive factors comprising a free energy input (3). Thus raise the temperature, T_t, of the inverse temperature transition above the physiological temperature and the matrix will swell to a tenfold increase in volume; lower the value of T_t to below physiological temperature and the matrix will contract to 45% of its swollen length. When the intensive variable is a change in chemical potential and the result is mechanical work, it appears that the chemical energy required to achieve a given amount of mechanical work is an order of magnitude more efficient for this ΔT_t mechanism of changing the temperature of an inverse temperature transition than for a mechanism based on charge-charge interaction (3). This new class of biomaterials is capable, therefore, of carrying out efficient free energy transduction.

Thus particular compositions of bioelastic materials have been shown to be biocompatible and transducible matrices and as they are polypeptides, i.e., protein-based polymers, it is to be expected that they would be biodegradable given sufficient time, e.g., a few weeks or months. Accordingly, it was somewhat surprising to learn that strips of 20 Mrad cross-linked poly(VPGVG), i.e., X^{20}-poly(VPGVG), could be implanted in the peritoneal cavity of rats for several months without visual evidence of degradation (4). What is retrieved from such intraperitoneal implants is the same clear transparent matrix that had been implanted, a matrix around which there had not been the formation of a fibrous capsule (1,4,5).

In the present report are the initial efforts to gain a measure of control over the rate of non-enzmatic degradation of bioelastic materials by utilizing chemical clocks both in the backbone structure and in the side chains. Seven polypentapeptides have been synthesized, i.e., I: Poly-(GVGVP); II: Poly-[0.9(GVGVP),0.1(GNGVP)]; III: Poly-[0.9(GVGVP),0.1(GVG$_c$VP)]; IV: Poly-[0.9(GVGVP),0.1(GNG$_c$VP)]; V: Poly-(VPGVG); VI: Poly-[0.9(VPGVG),0.1(VPG$_c$VG)]; and VII: Poly-[(VPGVG),0.1(VPG$_c$NG)], where N = Asn (asparagine) and G_c = glycolic acid, the α-hydroxy analog of glycine. When these polypentapeptides are placed in phosphate buffered saline (0.15 N NaCl, 0.01 M phosphate, pH 7.4), the Asn carboxamide at a rate depending on its adjacent residues will break down to carboxylate, i.e., Asp = aspartic acid = D (6), and the backbone ester resulting from glycolic acid incorporation in place of glycine will hydrolyze. Both of these phenomena can be followed by monitoring the temperature, T_t, of the inverse temperature transition. At physiological pH the breakdown of an ester or a carboxamide to a carboxylate anion raises the temperature of the inverse temperature transition because the carboxylate anion is so much more polar than either an ester or a carboxamide (7). An important difference in the polymers listed as I. through VII. above is that the ester hydrolysis results in fragmentation (degradation) of the polypentapeptides; whereas; the hydrolysis of the carboxamide results in the solubilization or, if cross-linked, in the swelling of the matrix.

EXPERIMENTAL

Polypentapeptide Syntheses

The syntheses of VPGVG and GVGVP have been previously described (8,9). The pentamers containing glycolic acid (G_c) and Asparagine (N) were synthesized as VPG$_c$VG, VPG$_c$NG, GNGVP, GVG$_c$VP and GNG$_c$VP by the solution phase method. It has been found in previous studies that the highest molecular weights were obtained when the pentamer permutation with Pro at the carboxyl terminus (GVGVP) and para-nitrophenol (ONp) were used for carboxyl activation (9). Here, however, the VPGVG permutation was used with Gly at the C terminus for pentamers substituted in position 3 with the glycolic acid residue and with 1-ethyl-3-dimethylaminopropylcarbodiimide (EDCI) as the polymerizing agent. The reasons for these changes were [1] with GVGVP permutation, glycolic acid at position 3 occurs at the N terminus which may be difficult to polymerize under normal conditions necessitating in these cases the use of the VPGVG permutations, and [2] at the end of the polymerization, the terminus ONp moieties were removed by base treatment with 1 N NaOH which would hydrolyze ester bonds present in the polymers. Moreover, our previous studies (1,10) indicate that using EDCI with Pro at the carboxyl terminus also gives equally good polymers which were identical in transition temperature, carbon-13 nuclear magnetic resonance (NMR) spectra, amino acid analyses and biological studies. When the VPGVG permutation is used, the value for T_t is often higher.

The first three pentamers, Boc-GNGVP-OH, Boc-GVG$_c$VP-OH and Boc-GNG$_c$VP-OH, were mixed with Boc-GVGVP-OH in a 1:9 ratio (Boc being t-butoxycarbonyl), and the last two pentamers, Boc-VPG$_c$VG-OH and Boc-VPG$_c$NG-OH, were mixed with Boc-VPGVG-OH. Each mixture was deblocked with trifluoroacetic acid, and a one-molar solution of the trifluoroacetate salt in dimethylsulfoxide was polymerized for fourteen days using EDCI as the polymerizing agent with 1-hydroxybenzotriazole and N-methylmorpholine as base. The reference polypentapeptides, poly(GVGVP) and poly(VPGVG) were also synthesized. The polymers were then each dissolved in water, dialyzed using 50 kD mol. wt. cut-off dialysis tubing and lyophilized. The polymers that were synthesized are listed in the Introduction. The purity of the intermediates and the final products was checked by thin layer chromatography, carbon-13 NMR spectroscopy and amino acid analysis. These polymers can be described with the general formula poly[f_V(GVGVP),f_X(GXGVP)], etc., where f_V and f_X are the mole fractions with $f_V + f_X = 1$. Based on the amino acid analyses, the more correct statement of the formulae for the polymers with mixed pentamers would be II: Poly-[0.92(GVGVP), 0.08(GNGVP)]; III. Poly-[0.85(GVGVP), 0.15(GVG$_c$VP)]; IV. Poly-[0.95(GVGVP), 0.05(GNG$_c$VP)]; VI: Poly-[0.9(VPGVG), 0.1(VPG$_c$VG)]; and VII: Poly-[0.94(VPGVG), 0.06(VPG$_c$NG)]. More details of peptide synthesis and product verification will be presented elsewhere.

Determination of T_t, the Temperature of the Inverse Temperature Transition

Each polymer was dissolved as a solution of 40 mg/ml in phosphate buffered saline (0.15 N NaCl, 0.01 M phosphate, pH 7.4). At 37°C, each sample forms a coacervate layer on the bottom of the tube. The value of the temperature, T_t, of the inverse temperature transition was determined from the development of turbidity as the temperature was scanned from below to above T_t. At each time interval, the temperature of the sample was lowered to dissolve the coacervate (the viscoelastic layer) on the bottom of the tube and an aliquot was taken for determination of T_t. The temperature profiles for turbidity development (aggregation) were determined on a Pye-Unicam, Model PU 8610 spectrophotometer equipped with a temperature control cell attached to a Neslab RTE110 temperature control circulation bath. The temperature was scanned at a rate of 30°C per hour and the turbidity measured at 400 nm. Representative temperature profiles for

turbidity formation are shown in Figure 1 for polymers I, III, VI and VII. The value of T_t is taken as the temperature at which half maximal turbidity is obtained. This is similar to a cloud point temperature.

RESULTS

The change in T_t with time in phosphate buffered saline is given in Figure 2A for each of the seven polypentapeptides. Polymer I, poly(GVGVP), shows no change with time indicating that the polypentapeptide backbone is stable to hydrolytic cleavage under these conditions. Also, this solution shows no significant change in pH with time. Poly[0.92(GVGVP),0.08(GNGVP)], polymer II, exhibits an increase of about 1.5°C in a period of five days. This is due to the breakdown of the asparagine carboxamide to carboxylate resulting in the aspartic acid residue. The ΔT_t for the conversion of Asn to Asp for $f_x = 1$ is 120°C. This value is obtained from the T_t-based hydrophobicity scale (3,7). Since $f_x = 0.08$, the expected shift for complete conversion of Asn of polymer II to Asp would be 9.6°C. At 40 mg/ml the concentration of pentamers is about 100 mM such that the concentration of Asn would be (0.08 x 100 =) 8 mM. With an initial ΔT_t of 0.36°C/day, the breakdown would be 8 x 0.36/9.6 = 0.30 mM $CONH_2 \rightarrow COO^-$ per day or a half-life of 18 days for the PGNGV sequence in physiological phosphate buffered saline (PBS).

It is apparent for polymer III, poly-[0.85(GVGVP), 0.15(GVG_cVP)], that the backbone ester is also unstable in phosphate buffered saline. In this case the result of ester hydrolysis is chain breakage and the formation of an α COO^- moiety. As there is a chain length dependence of T_t (2), as well as the dependence on formation of the polar carboxylate (3,7), an effort to estimate the rate of ester hydrolysis from the change in T_t is more complex. It is also apparent that the initial rate is faster; presumably, this is due to the drop in pH seen in Figure 2B as physiological PBS (0.01 M phosphate) does not have a sufficient buffering capacity to hold the pH above 7. Since f_x = 0.15 for polymer III, the concentration of esters for a 40 mg/ml solution of polypentapeptide would be about 15 mM. Now if the T_t of 170°C for the βCOO^- of Asp at $f_x = 1$ is assumed for the α COO^- and the Glu value for T_t of 25°C is taken for the backbone ester, then a ΔT_t of 145°C would be expected. The effect of forming carboxylates on breakdown of all of the backbone esters would be 0.15 x 145 = 22°C. Now the initial rate of change of T_t is 0.62°/day and (15 mM x 0.62/22 =) 0.42 mM/day would be a rate of breakdown neglecting the effect of decreasing chain size on T_t. This translates into a half-life of 25 days which, as decreased molecular weight is neglected, is expected to be a minimal half-life. For polymer VI, the ΔT_t/day (initial rate) was 0.62°C/day which, when correcting for the change exhibited by the reference polymer, translates to a half-life of 18 days.

For the compounded structures containing both side chain carboxamides and backbone esters, the initial ΔT_t values were 0.84°C/day for polymer IV and 2.13°C/day for polymer VII. What is apparent is that the modifications of the polypentapeptide provide significant and useful increases in the rate of degradation under conditions of physiological saline and phosphate.

DISCUSSION

Figure 2A demonstrates a differential rate of coacervate solubilization which is completed when T_t exceeds 37°C. As the carboxamide and ester to carboxylate breakdown presumably occurs at the interface of the coacervate and the overlying solution, the result is analogous to what would occur on the surface of a cross-linked matrix doped with a drug to be released. As the surface layers erode or swell with a tenfold increase in volume, the pharmaceutical would be released and the rate of release would be dependent on the half-life of the chemical clock that had been incorporated within the matrix.

Because bioelastic materials are capable of functioning as free energy transducers, cross-linked matrices can be designed to bring about drug release by controlling the rate of swelling with diffusional release of incorporated drug or by controlling the rate of contraction, for example, of an envelope to expel drug laden contents. In addition, bioelastic materials can function in drug delivery by the more traditional means of controlling the rate of degradation. Also, it is likely that desirable combinations of the two means could be designed.

Bioelastic matrices are capable of functioning by means of the first three of the four controlled release processes enumerated by Peppas and Korsmeyer, i.e., [1] diffusion controlled, [2] chemically controlled (degradation), [3] solvent-activated and [4] magnetically controlled (11). As a more general description, the transductional capacity of bioelastic materials allow that any of the five intensive variables (mechanical, thermal, chemical, pressure and electrical) may be used to control release with, for example, the chemical case having possibilities of both polymer-based and solvent-based mechanism with numerous ways of achieving an integrated diagnostic therapeutic pair (12).

ACKNOWLEDGMENTS

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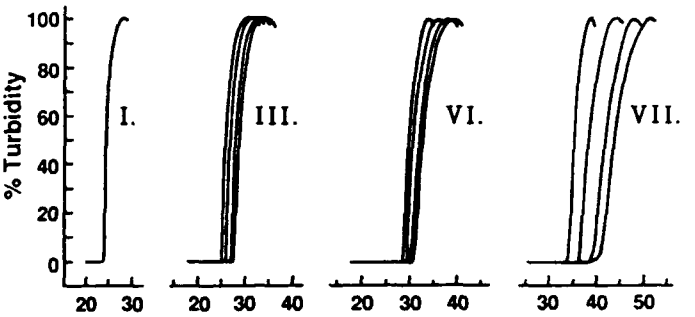

FIGURE 1

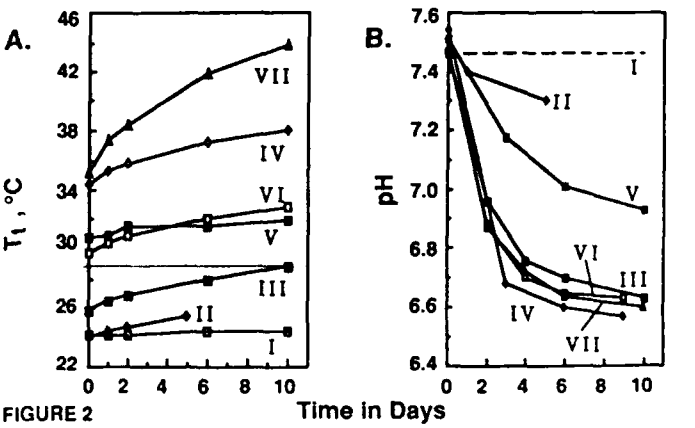

FIGURE 2

(To be filled in by Division)

Paper number as listed on program _____

[A.] DIVISION OF PMSE "Biotechnology & Bioactive Polymers" Symposium

[B.] TITLE OF PAPER DEVELOPMENT OF BIOELASTIC MATERIALS AS BIOCOMPATIBLE, TRANSDUCIBLE AND DEGRADABLE DRUG DELIVERY MATRICES

Time Required for Presentation _____

☐ Poster Presentation Preferred

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D.W.Urry+, D.C.Gowda#, C.Harris#, R.D.Harris+#, B.A.Cox#	+The Univ. of Alabama at Birmingham Laboratory of Molecular Biophysics, School of Medicine, VH300, Univ. Station, Birmingham, AL 35294-0019. #Bioelastics Research, Ltd. 1075 South 13th Street, Birmingham, AL 35205	☐ Yes ☐ No	☐ Yes ☐ No	☐ Chemist ☐ Chemical Engineer Other Molecular Biophysicist,Ph.D.

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DEVELOPMENT OF BIOELASTIC MATERIALS AS BIOCOMPATIBLE, TRANSDUCIBLE AND DEGRADABLE DRUG DELIVERY MATRICES. D. W. Urry,† D. C. Gowda,# C. Harris,# R. D. Harris†# and B. A. Cox#†, Laboratory of Molecular Biophysics, School of Medicine, The University of Alabama at Birmingham, VH300, UAB Station, Birmingham, AL 35294-0019, #Bioelastics Research, Ltd., 1075 South 13th Street, Birmingham, AL 35205.

The elastomeric polypeptide, poly(Val^1-Pro^2-Gly^3-Val^4-Gly^5), and its γ-irradiation cross-linked matrix are biocompatible and the matrix can be designed to swell or to contract in a manner to effect drug delivery by use of any of a number of intensive variables, that is, to perform free energy transduction as a means of achieving drug delivery. Furthermore, reported here are the first demonstrations that chemical clocks can be introduced to vary the rate of degradation and/or dissolution or to trigger transduction in physiological phosphate buffered saline. At the approximate concentration of one per fifty residues, the side chain carboxamide (of asparagine at position 4) and the ester (due to glycolic acid replacing glycine at position 3 or 5) break down to form carboxylates to effect dissolution of the viscoelastic matrix. The study of seven polypentapeptides indicates that the ester at position 3 is less stable than at 5 and the compounding of glycolic acid^3-asparagine^4 provides the most unstable linkage where complete dissolution is achieved within days whereas there is essentially no breakdown of the parent polypentapeptide under these conditions. Thus chemical clocks with a range of half-lives have been incorporated into poly(VPGVG) that can control the rate of degradation or of transduction to achieve drug delivery.

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#402

MEDICAL APPLICATIONS OF BIOELASTIC MATERIALS

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ABSTRACT

Repeating peptide sequences that occur in mammalian elastic fibers exhibit interesting properties of entropic (ideal) elasticity and of folding and assembling on raising the temperature. When correctly understood, these properties make it possible to design and synthesize elastomeric polypeptides capable of exhibiting additional properties and functions with both medical and non-medical applications.

For medical applications, it is first necessary to demonstrate biocompatibility. This has been done for the parent elastic protein-based polymer, poly(Val^1-Pro^2-Gly^3-Val^4-Gly^5) or poly(VPGVG), which exhibits an extraordinary biocompatibility as the polymer and as the γ-irradiation cross-linked elastomeric matrix, designated as X^{20}-poly(VPGVG). As cells do not adhere to this matrix and as no fibrous capsule forms around it when implanted, the matrix and other states of the material have potential for use in the prevention of post-operative, post-trauma adhesions. Specific studies underway include a contaminated peritoneal model using the rat, a strabismus surgery model using the rabbit eye, and just beginning a total artificial heart model as a bridge to transplantation using the calf.

Because cell attachment sequences can be introduced into the matrix which result in cell adhesion, spreading and growth to confluence, such modified elastomeric matrices become candidates for use in a wide range of possible tissue reconstructions. In general, the concept is to design a functional scaffolding for a particular tissue into which the appropriate cells can migrate and within which they function and remodel the prosthetic material into a natural tissue with slow degradation and removal of the temporary synthetic scaffolding.

These compliant, biocompatible matrices also have significant potential for use in drug delivery. This is made especially attractive because, in addition to being able to introduce chemical clocks that would control their rate of degradation as desired, they

can be designed to swell or to contract in response to a relevant chemical signal which may be associated with a particular diseased state.

This capacity to design the matrices to contract and relax in response to a chemical signal (i.e., to function in chemomechanical transduction) and even to carry out more diverse free energy transductions known to be important in living organisms suggests further dynamic roles in their potential medical applications.

INTRODUCTION

GENERAL BACKGROUND

Elastomeric Polypeptides that Increase Order on Increasing the Temperature: Bioelastic materials have their origins in repeating sequences that occur in the mammalian elastic fiber [1-3]. The most prominent repeating sequence is $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5)_n$ with n = 11 in bovine elastin [3]. High polymers of this sequence, poly(VPGVG), have been chemically and microbially synthesized [4-6]; they exhibit the interesting property of being soluble in water at low temperatures, below 25°C, but then aggregate into a more-ordered, viscoelastic state, called a coacervate, on raising the temperature to 37°C [7]. This process of increasing order on increasing the temperature is called an inverse temperature transition [8].

Matrices for the Conversion of Thermal Energy Into Motion: When the coacervate state is cross-linked, it forms elastomeric matrices with elastic moduli similar to those of the elastic fiber. When 20 Mrads of γ-irradiation are used to achieve cross-linking, the resulting elastic matrix is designated X^{20}-poly(VPGVG). A small sheet of elastic matrix is seen in Figure 1. The cross-linked matrix exhibits the inverse temperature transition behavior by contracting on raising the temperature to 37°C and swelling on lowering the temperature to 20°C. If a weight is hung on the swollen elastic matrix, the weight will be lifted on raising the temperature. Therefore, the cross-linked matrix can convert thermal energy into motion with the performance of mechanical work. This is thermomechanical transduction [8].

The Matrix Can Be a Thermally Controlled Superabsorbent: As the poly(VPGVG) in the coacervate state is more dense than water, it forms a viscoelastic layer on the bottom of the container. When the temperature is lowered, it dissolves and becomes molecularly dispersed throughout the volume of the container whatever its size. When the thermally contracted elastic matrix, X^{20}-poly(VPGVG) at 37°C or above, is cooled to 20°C or less, it swells to ten times the contracted volume. If the cross-links were fewer and the chains as long as necessary, it could swell to even greater volumes. Thus, the elastic matrix can be a thermally controlled superabsorbent.

Versatility of Composition and a Hydrophobicity Scale: Starting with this basic material, by design and syntheses, the parent bioelastic material can be altered to exhibit a wide range of physical properties with many potential applications. With properly chosen amino acid substitutions, the temperature of the inverse temperature transition, which is a hydrophobic folding and assembly transition, can be lowered, if the substitute residue is more hydrophobic, or it can be raised, if the substitute residue is less hydrophobic. The general formula for such substitutions is poly[f_V(VPGVG),f_X(VPGXG)] where f_V and f_X are mole fractions with $f_V + f_X = 1$ and X is any of the naturally occurring amino acids or chemical modifications thereof. A complete hydrophobicity scale of all of the naturally occurring amino acids and of ionized states and of chemical modifications thereof has thus been developed [8-10].

Accordingly, a wide range of compositions are available for adding specific functional capacities, and the transition temperature can be placed as desired within the range available to aqueous media.

Matrices for the Conversion of Chemical Energy Into Motion (Or a Chemically-Controlled Superabsorbent): When the matrix contains even a very few (2 to 4 per 100 residues) amino acid residues with ionizable side chains, increasing the degree of ionization can drive unfolding and decreasing ionization can drive folding (contraction). The matrix has thus been designed to convert chemical energy into the motion of contraction [11]. Also, of course, one can control the functional superabsorbency of such a matrix. Furthermore, because of the present level of understanding of the forces involved, it has been shown that proper design at nanometric dimensions can dramatically change the pKa of an amino acid side chain. For example, the pKa of a Glu residue has been shifted, by design, from 4.3 to 8.1 and the titration curve is relatively steep (positively cooperative) [12]. This means that an elastic matrix can be designed to swell or contract over any chosen, small (less than one pH unit) pH range.

A Change in Concentration of Salts or Organic Solutes Can Be the Chemical Energy to Drive Contraction: Increasing salt concentrations such as NaCl, NaBr, Na_2CO_3, Na_3PO_4 (in order of potency) can lower the temperature of the folding and assembly transition [13]. Accordingly, if the transition temperature were just above 37°C, addition of any of these salts will lower the transition temperature below 37°C and contraction will occur. The effect of organic solutes is more varied; a very small amount of sodium dodecyl sulfate will raise the transition temperature; guanidine hydrochloride and urea do so in order of decreasing potency. Ethylene glycol, glycerol, trifluoroethanol and dimethysulfoxide (at higher concentration) in order of increasing potency lower the temperature of the folding transition [13].

Introduction of Enzyme Specificity: Enzyme sites can be introduced into the sequence. When the cyclic AMP dependent protein kinase site of lysozyme [14], Arg-Gly-Tyr-Ser-Leu-Gly, i.e., RGYSLG, is introduced into the polypentapeptide, poly[30(IPGVG),(RGYSLG)] where I = Ile, the serine can be phosphorylated by the protein kinase and intestinal alkaline phosphatase can remove the phosphate [15-16]. Serine phosphorylation has a tremendous effect; the phosphate is more potent in raising the temperature of the folding transition than any other known chemical modification [17]. When at the correct intermediate temperature, one phosphate in 300 residues can completely unfold the protein-based polymer. Besides showing the dramatic effect of phosphorylation on hydrophobic folding, this result demonstrates the principle that reactive enzyme sites can be introduced such that the specificity of enzyme reactions can be used to drive between folded and unfolded states.

Introduction of cell Attachment Sites: As will be discussed in more detail below, the elastic, transparent matrix of Figure 1 does not support cell attachment, but addition of a cell attachment sequence such as GRGDSP converts the matrix to an excellent substrate for cell adhesion and for providing for cell growth to confluence.

BIOCOMPATIBILITY

From this brief general background, it is hoped that the depth of knowledge of this system is apparent and that the breadth of potential applications is also apparent given the properties outlined. For medical applications, however, biocompatibility must be demonstrated and this has been done for poly(VPGVG) and its γ-irradiation cross-linked elastic matrix, X^{20}-poly(VPGVG). The series of tests that have been run by the North American Science Associates, Inc. (NAmSA®) and the favorable results are given in Table I. Quoting from the abstract of the publication that reviewed the results

"Thus, this new elastomeric polypeptide biomaterial exhibits an extraordinary biocompatibility" [18].

MEDICAL APPLICATIONS UNDER DEVELOPMENT

Given the properties of the bioelastic materials noted above, a number of medical applications that are now under development can be mentioned. First there are several animal models being used to determine the efficacy of the bioelastic materials as barriers in the prevention of unwanted adhesions that result as a consequence of surgical procedures and other trauma. Second, the bioelastic sheets are also being studied for their capacity to be refractory to cell attachment and then when appropriately modified to be converted to cell attachment matrices wherein the cells can attach and grow to confluence. This is in preparation for use of the materials in tissue reconstruction. And finally, in a more direct utilization of some of the unique physical properties of various forms of energy conversion, some rudimentary efforts at designing the elastic matrices for drug delivery have been initiated.

PREVENTION OF ADHESIONS

There are 30 million surgical procedures performed annually in the U. S. and an equivalent number in Europe. A serious postoperative complication in all of these is the formation of adhesions. It has been estimated that a fourth of these could be expected to have an improved outcome if a material were available to prevent adhesion formation. In developing uses for bioelastic materials, three animal models are being considered. The first is a contaminated peritoneal model using the rat for which there is now substantial data [19]. The second is a rabbit eye model for strabismus surgery in which a sleeve of the bioelastic material is placed around the superior rectus muscle in an effort to prevent the postoperative scarring that can defeat the objective of the corrective procedure [20]. And the third, for which studies are just beginning, utilizes the bioelastic material in the calf under cardiopulmonary bypass to prevent adhesion to the lung, and in the artificial heart to wrap the Dacron® vascular graft used to conduct the blood from the pulmonary artery and the aortic valve to their remnants and to cover the Dacron® velour which itself covers the outer surface of the atrial cuffs for the purpose of preventing adhesion of the left atrium to the pericardial wall and of the right atrium to the lung. The objective is to improve the usage of the total artificial heart as a bridge to transplantation.

In the Contaminated Peritoneal Model [19].

In this rat model, the abdominal wall is scraped with a scalpel until bleeding ensues; a loop of bowel is loosely secure over the injury with a loop of suture; the bowel is then punctured at the site apposition four times with a 20 gauge hypodermic needle, and the intestine is milked to effect leakage of contents and contamination of the site. In the controls, all of 29 control animals exhibited adhesions. There were two sets of test animals depending on the technique used for sterilization of the bioelastic sheets. For 30 animals, steam sterilization was used, and for 29 animals ethylene oxide gas sterilization was used. After positioning the test material between wall and intestinal loop, which positioning utilized the loop of suture loosely holding the bowel in apposition to the wall, the animal was closed. At seven days, the loop of suture was cut. At fourteen days, the animals were sacrificed and the adhesions were graded 0, 1, 2, or 3 [21]. Grade 0 was the absence of adhesion; grade 1 was a single band of adhesion comprised of omental fat between omentum and wall, which adhesion offered no resistance to separation; grade 2 was a fibrous band of adhesion between viscera and abdominal wall requiring moderate force to separate; and grade 3 was adhesion requiring sharp dissection to separate. Grades 0 and 1 are considered to be

insignificant adhesions whereas grades 2 and 3 are viewed as significant adhesions. For the gas sterilized material, 59% of the animals exhibited no adhesions; 21%, grade 1; 10%, grade 2; and 10%, grade 3. For the steam sterilized material, 40% of the animals exhibited no adhesions; 16%, grade 1; 13%, grade 2; and 30%; grade 3. Of the total of seven animals that exhibited grade 2, adhesions five were due to a tear in the matrix through which the adhesions passed. In the cases of the grade 3 adhesions, the bioelastic materials was completely encapsulated, but the bioelastic material was not adherent to the fibrous connective tissue; rather, it could be easily removed with tissue forceps.

An interesting example is shown in Figure 2 where a band of adhesion formed around the bioelastic matrix. The X^{20}-poly(VPGVG) is seen as a transparent elastic matrix after two weeks of implantation. There is no fibrous capsule of any kind that surrounds the matrix. There is no inflammation where the matrix has been in contract with the abdominal wall for two weeks. The elastic matrix has been seen to remain clear without fibrous capsule and undergraded after up to six months in the peritoneal cavity. The results of this study involving 88 animals indicate that X^{20}-poly(VPGVG) is an effective barrier against adhesion formation in this surgically-induced, contaminated wound model.

In the Strabismus Surgery Model [20].

Strabismus is a disorder in which the eyes cannot simultaneously focus on the same point. The positioning of each eye is due to four rectus muscles. In strabismus surgery a rectus muscle is detached and repositioned to improve alignment of the eyes. When adhesions occur following strabismus surgery or retinal detachment surgery involving rectus muscle, sclera of the eye or conjunctiva, eye movement can be severely restricted and misalignment can also result. The use of a number of materials has been attempted in the past with such limited success that they are now largely abandoned.

Preliminary studies have now been carried out using two formulations of the bioelastic material, X^{20}-poly(VPGVG) and X^{20}-poly[3(VPGVG),(VPGFG)] where F is phenylalanine. The model utilized the rabbit eye in which the superior rectus muscle was detached at its insertion; a small patch of sclera was removed underlying the muscle; the muscle capsule in apposition to the site of sclerectomy was removed, and the muscle was reattached at its original insertion site. In this control, the sclera and the muscle were tightly adhered at one week. When sleeves of X^{20}-poly(VPGVG) were used in three animals, neither inflammation nor adhesion occurred. When sleeves of X^{20}-poly[3(VPGVG),(VPGFG)] were used, no inflammation occurred but a glistening fibrous capsule formed around the sleeve which could limit eye movement. The improvement desired for the use of X^{20}-poly(VPGVG) would be to have a material which would degrade and disappear. Work in this direction is underway as will be noted below in the drug delivery application.

In the Total Artificial Heart as a Bridge to Transplantation.

In 1989 more than 400,000 open-heart procedures were performed in the United States with a substantial percentage of these being reoperations necessitated by either progression of the coronary artery disease, graft stenoses or occlusion. These secondary procedures involve far greater risk in large part due to the formation of postoperative adhesions [22].

One of the major contributing factors in the development of adhesions following open-heart surgery results from the inability to close the pericardium without compromising the procedure due to increased heart size resulting from

cardiopulmonary bypass(CPB)-induced distention. When the native pericardium is not closed, adhesion formation between the epicardium and sternum can be so massive that reoperation is extremely difficult and with significant risk of rupturing the heart.

With the increasing number of patients requiring reoperation and with the hope that total artificial hearts eventually will allow patients to survive until natural hearts become available, there is significant need for the development of a pericardial substitute which will allow isolation of the heart and prevent adhesion formation.

Structurally, the pericardium is a sac of dense connective tissue surrounding the heart. In addition to the heart, it contains the roots of the great arteries and veins. The fibrous pericardium is lined on its inner surface by serous pericardium which consists of parietal and visceral layers. Between the two layers a narrow space, the pericardial cavity, contains a thin film of serous fluid. The two layers slide freely against each other and are continuous where the great vessels pierce the fibrous pericardium. Histologically the serous pericardium consists of another layer of flattened mesothelial cells resting on a layer of connective tissue. These mesothelial cells produce pericardial fluid which is passed into the pericardial cavity.

A number of different types of materials have been tested for use as pericardial substitutes. These include, silicone membranes, polyurethane, fascia lata, polytetraflouroethylene (Gore-Tex) patches, bovine and porcine xenographs treated with glutaraldehyde, siliconized Dacron® and dura mater [22].

Although some materials appear to have been successful in preventing adhesions in animal models where the artificial pericardial material was implanted using thoracotomy or sternotomy without cardiopulmonary bypass, the results on humans have been far less successful. A possible explanation for the difference in the results from animal experiments and human clinical trials may be due to the fact that the animal experiments did not involve cardiopulmonary bypass (CPB). Evidence indicates that CPB itself causes damage to the pericardium and epicardium in humans which may impair the fibrinolytic activity of native pericardium [22].

There is a keen awareness of the necessity and need for a material that could be used in all surgeries to minimize the amount of adhesions and fibrous connective tissue (scarring) to avoid the adhesions which induce complications such as bowel obstruction, and constrictive pericarditis, the severity of hemorrhage in redo operations, and the horrors of severing previously implanted coronary artery vascular grafts. Experiences of Olsen and colleagues in developing the total artificial heart led them to look at some membranes that might be used to eliminate the adhesions and facilitate reoperations such as in replacement of the devices as well as explantation preceding cardiac transplantation. Smooth glutaraldehyde-treated pericardial allografts were used, without success [23].

In studies just now getting underway, the Utah team is to test X^{20}-poly(VPGVG) and other formulations at three sites in the calf under cardiopulmonary bypass. Adhesion problems at these sites have been considered one of the primary factors in relating results from animal studies to the human experience, according to Gabbay [22]. The plan is to place a sheet of the test materials over the lung and beneath the pleural suture line after the excision of the fifth rib in the right thorax, by tacking the four corners of the sheet, fixing the materials between the pleural suture line and the lung, an area ubiquitous with adhesions following total artificial heart implantation in the calf.

The second mode will be to wrap the Dacron® vascular graft used to conduct the blood from both the pulmonary artery and the aortic valve to the remnant aorta and pulmonary artery.

This vascular graft comes in contact with the lung, where large adhesions form, making explant of the device very difficult. The third test area will be to cover the Dacron® velour covering that is placed onto the exterior of the artificial atrial cuffs, that also adhere to the surrounding tissue, both to the pericardial wall on the left atrium and to the lung on the right atrium. These membranes will be placed in animals designed to survive from one to eight months, at which time the animal will carefully be autopsied, and the degree of scarring and adhesions carefully evaluated and compared to the scarring in the control calf.

These studies to enhance the use of the total artificial heart as a bridge to transplantation add an important dimension to the use of bioelastic materials in the prevention of adhesions and one in which the rate of degradation of the material need not be increased.

DEVELOPMENT OF ELASTIC MATRICES FOR CELL ATTACHMENT AS A STEP TOWARD TISSUE RECONSTRUCTION

It is becoming increasingly apparent in order for cells to function properly in a tissue that they require proper attachment to the extracellular matrix. The attachment occurs through receptors in the cell membrane called integrins which bind to specific peptide sequences within proteins comprising the extracellular matrix. The most celebrated cell-attachment sequence is the RGD-sequence of fibronectin to which fibroblasts and other cells attach [24-28]. The role of the attachment is in part to transmit tensional forces to which a tissue is subjected through the cell membrane to intracellular components. The changed stresses on the intracellular components such as the cytoskeleton result in chemical signals for the cell to function in such a way as to reinforce the tissue to withstand the applied stresses. The chemical responses within the cell include "ion transport, release of chemical second messengers, protein synthesis, secretion, and even expression of specific genes" [29]. Evidence of this was provided some years ago by Glagov and colleagues in which it was found that vascular wall cells when attached to vascular elastic lamina would turn on production of extracellular macromolecules in response to cyclic stretching in an effort to rebuild the natural vessel wall [30-32]. This has more recently been demonstrated in an elastic vascular prosthesis by the Dutch group [33,34]. The challenge for the vascular prosthesis is to have a functional elastic vessel that would serve as a temporary functional scaffolding that would degrade as the natural vessels were regenerated.

As will be shown below, functional cell attachment sequences can be introduced into the elastomeric polypeptide to form matrices that provide for cell attachment. It may also be noted that the physical properties demonstrated in these matrices by virtue of their capacity to undergo inverse temperature transitions demonstrate a likely mechanism whereby stretching of protein could result in chemical responses such as dephosphorylation/phosphorylation, pH changes, etc. For example, these matrices when appropriately designed exhibit stretch-induced pKa changes, i.e., stretch-induced changes in pH [35]. To our knowledge, only the bioelastic materials containing cell attachment sequences can provide for both cell attachment and the dynamic elastic response necessary to signal the cell to function in a manner appropriate to the tissue of residence. These properties unique to bioelastic materials can be expected to secure a role for elastomeric polypeptide matrices in tissue reconstruction and engineering.

Cell Adhesion in the Absence and Presence of Serum [36,37].

When synthesizing the elastomeric polypeptide, it is often an advantage to polymerize the GVGVP permutation with Pro at the carboxyl terminus rather than VPGVG with Gly at the terminus. This, of course, gives the same polymers differing only in the particular residues that begin and end the several hundred residue sequence. Thus, a

polymer containing 20 GVGVP pentamers to one cell attachment sequence GRGDSP would be written poly[20(GVGVP),(GRGDSP)] and the cross-linked matrix X^{20}-poly(GVGVP) is essentially identical to X^{20}-poly(VPGVG).

First to be discussed as a baseline or control for the cell attachment studies are X^{20}-poly(GVGVP) and a structurally related elastomeric X^{20}-poly(GGAP) [37]. As seen in Figure 3, human umbilical vein endothelial cells(HUVEC) attach to neither elastic matrix when the appropriate culture medium contains 0.1% bovine serum albumin(BSA). When the culture medium contains 20% fetal bovine serum(FBS), there is poor cell adherence to X^{20}-poly(GVGVP) without capacity for growth to confluence but there are still no cells whatever adherent to X^{20}-poly(GGAP). When ligamentum nuchae fibroblasts(LNF) are used as in Figure 4 again only poor adherence is observed with X^{20}-poly(GVGVP). This is interpreted to suggest that some serum proteins can weakly adsorb on the surface of X^{20}-poly(GVGVP) but not on the less-hydrophobic X^{20}-poly(GGAP). One implication of these results is that X^{20}-poly(GGAP) could possibly provide an even better barrier for adhesion prevention than X^{20}-poly(GVGVP) for those surgical procedures where there is much bleeding.

When using the X^{20}-poly[20(GVGVP),(GRGDSP)] and low-density platings of 1×10^4 cells/ml, the cells are seen to have attached and grown to confluency at three days (See Figure 5) [36]. These bioelastic surfaces appear to be equivalent to fibronectin coated surfaces in their ability to support cell attachment and growth to confluency.

Cell Receptor Specificity.

In the process of further characterizing the X^{20}-poly[20(GVGVP),(GRGDSP) cell attachment matrix, the interaction with blood platelets was considered. The blood platelets did appear on the surface, but did not spread or show signs of being activated, and could be rinsed off. Since platelets have the fibronectin receptor, this prompted characterization of the receptor that was responsible for fibroblast and endothelial cell attachment. As shown in Figure 6, using a series of cell adhesion inhibiting peptides [39], it is not the cell membrane receptor that binds to fibronectin that is responsible for the attachment of human umbilical vein endothelial cells(HUVEC) to the bioelastic matrix but rather the vitronectin receptor [38] in which the sequence recognized is RGDV [40]. The same is the case for ligamentum nuchae fibroblasts. This raises the attractive possibility for a vascular graft material which would encourage endothelial cell coverage of the intima without platelet activation.

A Step Toward an Artificial Pericardium.

The capacity to design elastomeric matrices that support cell attachment and growth and possibly remodeling of the matrix, the interest in developing a material to prevent adhesions following open-heart surgery, and the associated special problems of cardiopulmonary bypass-induced distention of the heart with the resultant inability to close the pericardium, combine to raise the possibility of developing an artificial pericardium. The elastic artificial pericardium would provide the added area required to cover the distended heart; it would contain cell attachment sites for mesothelial cells; and suitable fibrinolytic activity could be attached to the serosal surface. It could be designed to contract as the heart size returned to normal, and it is anticipated that it could be remodeled into natural pericardium.

There are, of course, numerous tissue reconstruction or tissue engineering applications, that may be considered, and these will be pursued as the stimulation of interested parties, research and development monies and time allow. It is also apparent

that these bioelastic matrices to which cells can attach, being both optically transparent and stretchable, would have many uses in basic research on cellular functions.

DEVELOPMENT OF DRUG DELIVERY DEVICES

Current Perspective on Drug Delivery. There is an ongoing interest in the development of materials for use in drug delivery systems both in the clinical and pharmaceutical communities. The clinical interest stems from the need for delivery stems for thereapeutic agents which can improve both the safety and efficacy of existing therapeutic agents, increase control over release patterns, provide for diversity in the use of existing agents and provide a means of delivering complex agents such as proteins in their active state. The pharmaceutical industries interest stems from an interest in gaining broader uses for existing agents.

Korsmeyer and Peppas [41] developed a classification system for controlled-release systems based on the mechanism which controls the release of the agent. The most common mechanism is Diffusion-controlled of which there are two types, (1) the Reservoir type in which the bioactive agent forms a core surrounded by an inert diffusion barrier and (2) the Monolithic type in which the active agent is dispersed or dissolved in an inert polymer. The second class consists of Chemically-controlled systems which utilize bioerodible or pendant chain systems. The advantage of bioerodible (or biodegradable) systems is that the device is eventually absorbed by the body and thus need not be removed. The third class consists of the Solvent-controlled system. In this case the agent is dissolved or dispersed in a polymeric matrix through which it cannot diffuse. As solvent penetrates the matrix, the polymer swells allowing the agent contained in it to diffuse. As solvent penetrates the matrix, the polymer swells allowing the agent contained in it to diffuse through the polymer. The fourth and final class consists of the Mechanically-controlled systems in which external forces control the release of therapeutic agent.

According to Lloyd [42], properties exhibited by the ideal macromolecular drug carrier would include the following: adequate drug-loading capacity, retention of water solubility when drug-loaded, molecular weight high enough to prevent glomerular filtration, but low enough to reach all cell types, unmodified carrier not captured by adsorptive pinocytoses, a stable carrier-drug linkage in body fluids but degradable in the lysosome, slowly biodegradable in extracellular compartment or degraded in the lysosome, non-toxic, non-immunogenic and generally biocompatible.

A large number of both synthetic and natural polymers have been studied for possible application in drug delivery. According to Ranade [43], the greatest advantage of the synthetic polymers is the wide choice available. Two promising synthetic polymers which have been developed are polyvinylpyrrolidone (poly VP) and N-(2-hydroxypropyl)methacrylamide (poly HPMA), with the latter having the advantage of being easily derivatized. The natural polymers have the advantage of being biodegradable. Among the natural polymers studied are poly(lactic acid), copolymers of DL-lactic acid and glycolic acid, and copolymers of gluconic acid and α-ethyl-L-glutamate.

Bioelastic Materials Add New Dimensions to Drug Delivery [44,45].

Molecular (Bioelastic) Machines as Drug Delivery Vehicles. A molecular machine is a macromolecular construct that can convert energy from one form, or one location, to another. The living organism may be described as the synergistic integration of functionally diverse molecular machines [13]. Biology's molecular machines are proteins. The bioelastic materials are protein-based polymers that can be designed to carry out all of the energy conversions recognized in living organisms [8,13] except

light driven processes. Studies to demonstrate light-driven folding and unfolding are in progress. Several free energy conversions for which there is now data to have demonstrated the conversion or the feasibility of the conversion are given in Figure 7.

A particularly recognizable energy conversion is the conversion of chemical energy into useful mechanical work. This is the chemomechanical transduction arrow of Figure 7. When the chemical energy is provided by the chemical alteration of the diseased state, drug delivery can be the useful mechanical result. When there is a difference in the concentration of chemical between the normal state and the diseased state, the bioelastic material could be designed to contract or to swell, either of which, depending on the vehicle design, could effect drug release. If the bioelastic materials were the envelope surrounding contents which included the drug, a chemically triggered contraction could result in expulsion. If the drug were in a monolith slab, a chemically-induced swelling could result in diffusional release. This integration of chemical-control and mechanical-control in a single device adds a new dimension to the Korsmeyer and Peppas classifications [41].

All of the arrows ending at the mechanical apex of Figure 7 originate at energy sources that could be used for mechanically-effected drug delivery. The free energy sources involve as the intensive variable a change in pressure, a change in temperature, a change in chemical potential (i.e., a change in the concentration of a chemical), and a change in electrochemical potential (i.e., a change in the oxidative state of a redox moiety attached to the protein-based polymer). At the chemical apex alone, there is all of the diversity of the chemical changes that was noted in the introduction. The changes could be intrinsic to the polymer as in a change in the degree of ionization, a change in the state of phosphorylation, etc. The changes could be extrinsic to the polymer as in the changes in concentrations of salts and organic solutes. At the electrical apex there are all of the chemical moieties that could function as redox couples, and these could be prosthetic groups such as the nicotinamides or flavines that can be oxidized or reduced by a relevant enzymatic activity. Thus, there can be designed many bioelastic constructs that could utilize many unique and relevant energy sources with which to achieve drug delivery. These free energy transductions are reviewed in a general article entitled "Free Energy Transduction in Polypeptides and Proteins Based on Inverse Temperature Transitions" [8]. Some of the specific constructs and the mechanochemical couplings are considered in more detail elsewhere for drug delivery [44,45]. Here, two more elements will be considered: control of the vehicle size and control of vehicle degradation.

Control of Vehicle Size.

Lloyd [42] in listing the properties for an ideal macromolecular drug carrier included the property of molecular weight as high enough to prevent glomerular filtration but low enough to reach all cell types. Studying the early events of the thermally-elicited hydrophobic folding and assembly, that is, the early aggregational steps of the temperature transition, using elastic- and quasi-elastic light scattering [46,47], it has been possible to find conditions for controlling aggregate size. The particle sizes can be set depending on temperature, concentration and time with radii ranging from 50 nm to 500 nm. In particular, the light scattering evidence indicates that particles with diameters centered at 200 nm can be stabilized. Now, if these could be fixed by cross-linking at such a size; they could be loaded with drug by thermal swelling followed by a contraction to trap the drug dispersed within the nanosphere. Such a size could be expected to be too large to pass through normal vasculature but would be small enough to pass through diseased vasculature. If the correct ionizable function could be designed within the particle such that the particle could be induced to swell in response to a change in pH due to the diseased state, for example, then the particle could be expected to escape the vasculature into the diseased tissue and to swell for a more rapid release of

the drug at those sites with the altered pH.

With the proper design of the bioelastic material, it appears possible to set the pKa of a functional side chain as desired. For example, by properly designing the protein-based polymer it has been possible to shift the pKa of a Glu residue from 4.3 to 8.1 [12]. The sequence that achieves this is poly[GEGFP GVGVP GVGFP GFGFP GVGVP GVGFP] where E is Glu and F is Phe. Related studies are underway to determine the magnitude of shifts that may be achieved for Asp(D), Lys(K), His(H) and Tyr(Y). In addition, it is relevant to note, as shown in Figure 8, that the titration curves in such designs for shifting the pKa can be very steep such that a change in pH of a fraction of a pH unit can effect the change from contracted to swollen or the reverse, i.e., the change required for complete drug release. Finally, it is expected that the change in volume of such a designed nanoparticle could be a factor of ten or more.

Control of Vehicle Degradation [28].

As reflected in the reviews of Korsmeyer and Peppas [41], of Lloyd [42] and of Ranade [43], degradation, whether due to bioerosion, biodegradation, or hydrolytic breakdown, is a useful means with which to control drug delivery, and it removes the delivery vehicle. But we have seen above that free energy transduction can be used in bioelastic materials to control drug release. In addition, the swollen bioelastic matrix would be expected to degrade, as it would be more accessible to proteolytic digestion than the contracted matrices that have been used in the prevention of adhesion models discussed above.

As has been discussed elsewhere, chemical clocks can be introduced in to the bioelastic matrix that would control the rate of swelling. The chemical clock could be in the side chain as, for example, an asparagine or a glutamine whose carboxamide can convert to carboxylates with half-lives depending on the sequence in which they are found ranging from days to decades [49]. This could trigger the swelling for drug release and for removal by enzymatic degradation. The chemical clock could also be in the backbone such as when replacing a glycine residue by a glycolic acid residue. In this case, hydrolytic cleavage would result both in drug release and in polymer breakdown.

The time dependence of the breakdown of asparagine(N) to aspartic acid(D) and of the hydrolysis of the glycolic acid(G_c) residue in the polypeptide backbone can be followed by the increase in the temperature for the inverse temperature transition [48]. This is shown in Figure 9 for the protein-based polymers. I: poly(GVGVP), the control; II: poly[0.9(GVGVP), 0.1(GNGVP)]; III: poly[0.9(GVGVP), 0.1(GVG_cVP)]; IV: poly[0.9(GVGVP), 0.1(GNG_cVP)]; V: poly(VPGVG), another control; VI: poly[0.9(VPGVG), 0.1(VPG_cVG)], and VII: poly[0.9(VPGVG), 0.1(VPG_cNG)]. By the experimental design, these are hydrolytic cleavages that are occurring at the surface of the viscoleastic, coacervate state in physiological phosphate buffered saline at 37°C. From the increase, in the transition temperature, polymer III has been estimated to have a half-life to complete dissolution of 25 days. The half-life for polymer VII is much shorter, and that for polymer I, a control, is essentially infinite. Thus, with this initial demonstration of two simple chemical clocks a wide range of half-lives can be obtained.

SUMMARIZING COMMENT

This short review presents some of the medical applications that are under development. The very brief preface was to make apparent the depth of our understanding of the science underlying these materials that are capable of exhibiting inverse temperature transitions. This work is reviewed extensively elsewhere [8]. The medical applications that were discussed and the underlying science are intended to demonstrate the breadth and potential that these new materials have.

ACKNOWLEDGMENT

This work was supported in part by Contract No. N00014-89-C-0282 and Contract No. N-00014-90-C-0265 from the Naval Medical Research and Development Command, Office of Naval Research, and by grant HL-29578 from the National Institutes of Health.

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FIGURE LEGENDS

Figure 1. Small sheet of 20 Mrad γ-irradiation cross-linked poly(GVGVP) designated as X^{20}-poly(GVGVP). (Note that in the polymer GVGVP is equivalent to VPGVG). This bioelastic matrix is optically transparent; it is elastic; it exhibits a thermally-driven contraction capable of doing useful mechanical work; and it exhibits salt-driven contractions capable of driving, for example, drug delivery.

Figure 2. View of the contaminated peritoneal animal model for studying the efficacy of a material to function as a barrier to prevent of postoperative adhesions. The transparent elastic sheet of X^{20}-poly(GVGVP) is seen lying against the abdominal wall and a small strip of adhesion has grown around the bioelastic matrix which was positioned between the injured abdominal wall and the apposed punctured bowel. (Note that there is no inflammation of the wall, and that the bioelastic material shows no signs of degradation). In the control animals, adhesions occurred 100% of the time in this rat model. With the bioelastic materials interposed, no adhesions occurred in 50% of the animals. Overall, for the gas sterilized matrix, either no adhesions or insignificant adhesions occurred in 80% of the 29 test animals. Submitted for publication.

Figure 3. Cell attachment studies of human umbilical vein endothelial cells(HUVEC) on either X^{20}-poly(GVGVP) or X^{20}-poly(GGAP) with either 0.1% BSA(bovine serum albumin) or 20% FBS(fetal bovine serum) added to the culture medium. Poor cell adhesion is observed on X^{20}-poly(GVGVP) only in 20% FBS. No cell adhesion is observed on X^{20}-poly(GGAP)

Figure 4. Studies on the adhesion of ligamentum nuchae fibroblasts(LNF) to either X^{20}-poly(GVGVP) or X^{20}-poly(GGAP). Again, only X^{20}-poly(GVGVP) is seen to support cell adhesion but the cells are poorly adhered and are not well-spread. Again, X^{20}-poly(GGAP) is seen to support no cell attachment whatever. This suggests that X^{20}-poly(GGAP) could be a better matrix for the prevention of postoperative adhesions even than X^{20}-poly(GVGVP) in operations where there is much bleeding.

Figure 5. Matrices of X^{20}-poly[20(GVGVP),(GRGDSP)] on which low density platings of bovine aortic endothelial cells (A) and ligamentum nuchae fibroblasts (B) are seen to have grown to confluence at three days (C) and (D), respectively. The endothelial cells are very well-adhered with the capacity in a parallel-plate flow chamber to remain attached and to align with high fluid shear stresses of 30 dynes/cm^2 (E. A. Sprague, unpublished data). Normal fluid shear stress values for arteries are 15-20 dynes/cm^2. This figure was reproduced with permission from [36].

Figure 6. Cell attachment studies on bioelastic matrices containing the GRGDSP cell attachment sequence from fibronectin. On the right-hand side are peptides that were introduced at 1 mM concentrations in the medium to assess their capacity to compete at the cell receptor and inhibits cell adhesion [39]. The second and third columns are fibronectin and vitronectin coated tissue culture plastic, respectively. From the studies, it is concluded that the cells are binding to the GRGDSP-containing bioelastic matrix utilizing this vitronectin receptor. Reproduced with permission from [38].

Figure 7. Potential free energy transductions utilizing bioelastic materials (elastic protein-based polymers). Designed elastic protein-based polymers have been synthesized which have demonstrated thermomechanical, chemomechanical and baromechanical transduction, and which have demonstrated the feasibility for electromechanical transduction. These all utilize inverse temperature transitions exhibited by the bioelastic materials. Reproduced with permission from [8].

Figure 8. Acid base titrations (dashed curves) of a bioelastic polymer poly[0.75(GFGVP), 0.25(GEGVP)] which exhibits hydrophobicity-induced pKa shifts and polymethacrylic acid which exhibits charge-charge repulsion-induced pKa shifts. In both cases, the chemical couple is (COOH/COO$^-$). The solid curves are the theoretical shapes for the titration of a weak acid. The essential point of the data is that the titration for the bioelastic polymer is steep exhibiting positive cooperativity and requiring only a small pH change to complete the titration, whereas the titration of polymethacrylic acid is broad exhibiting negative cooperativity and requiring a much larger pH change to complete the titration. As a change in proton concentration, a ΔpH, is a change in chemical potential and as the product of the change in chemical potential times the change in moles of titrated carboxyls is the chemical energy, it is apparent that a process utilizing the chemical energy would be more efficient for the bioelastic material. Reproduced with permission from [8].

Figure 9. Use of the change in the temperature of the inverse temperature transition, T_t, with time to evaluate the rate of dissolution of bioelastic polymers I through VII as defined and discussed in the text. Reproduced with permission from [48].

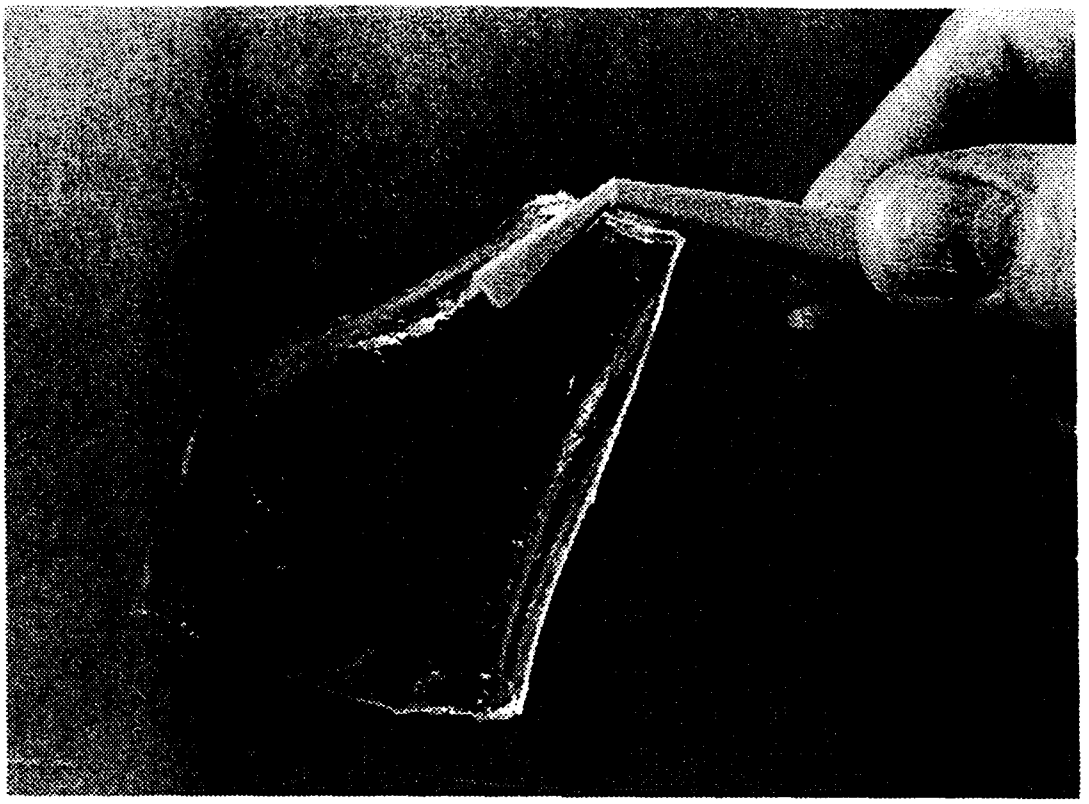


Figure 1

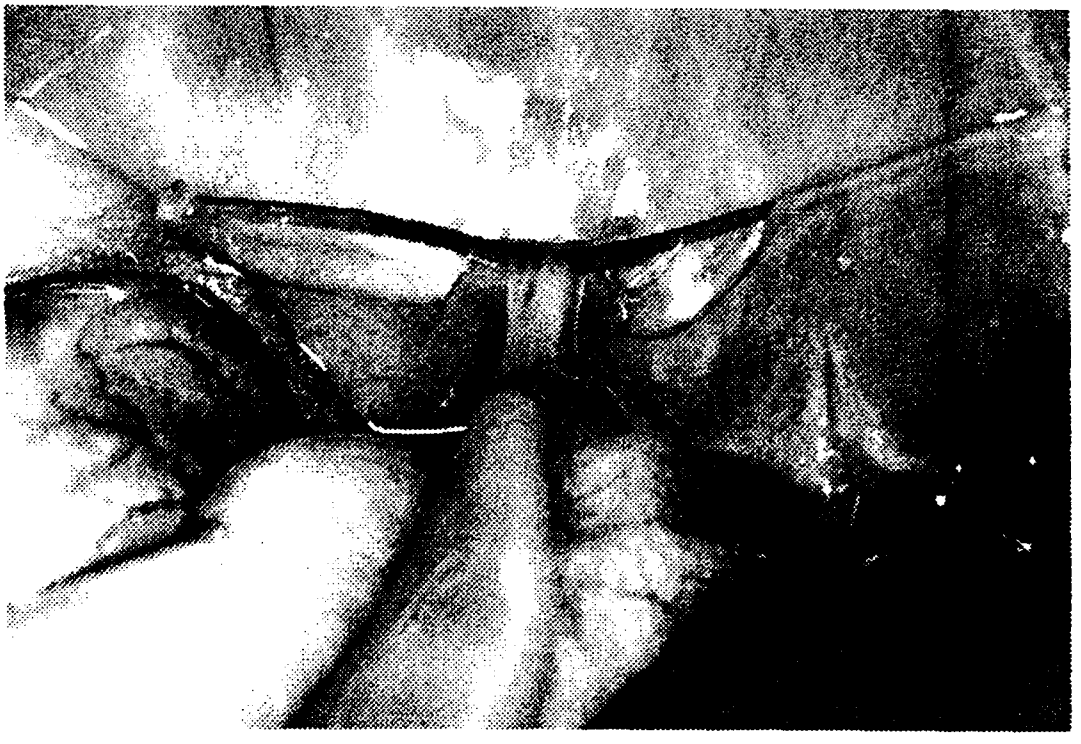
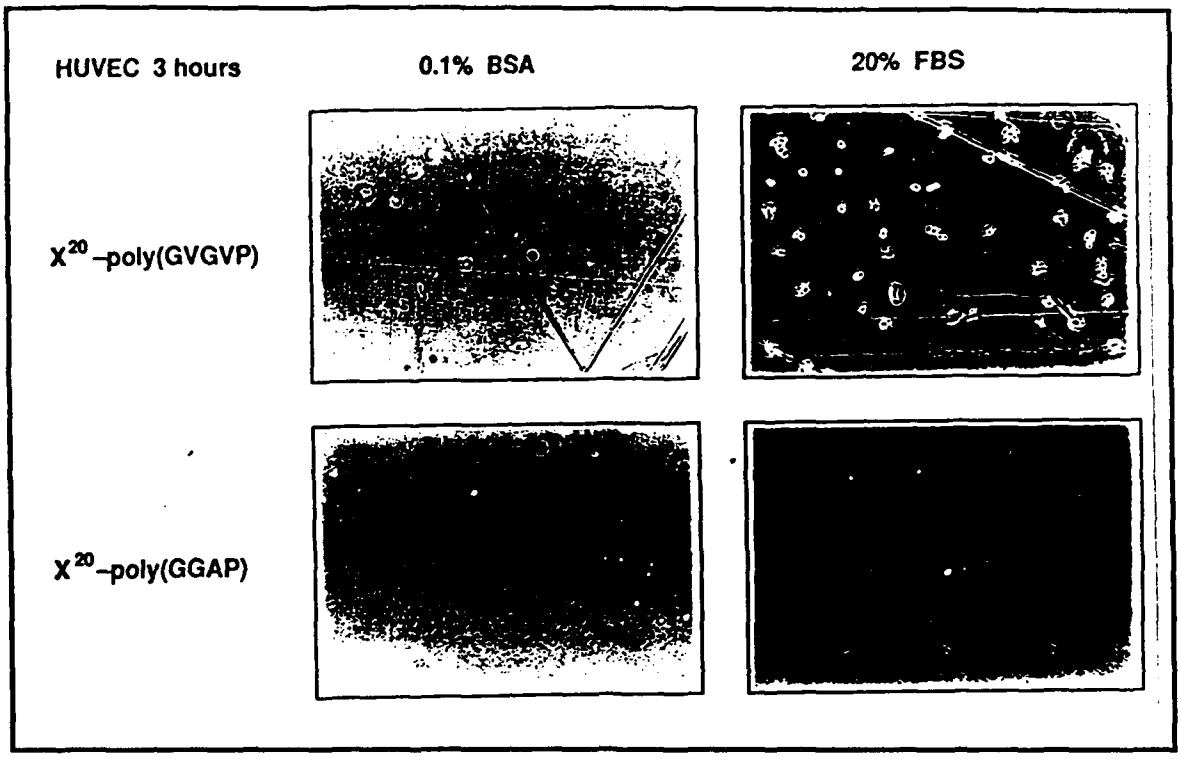


Figure 2



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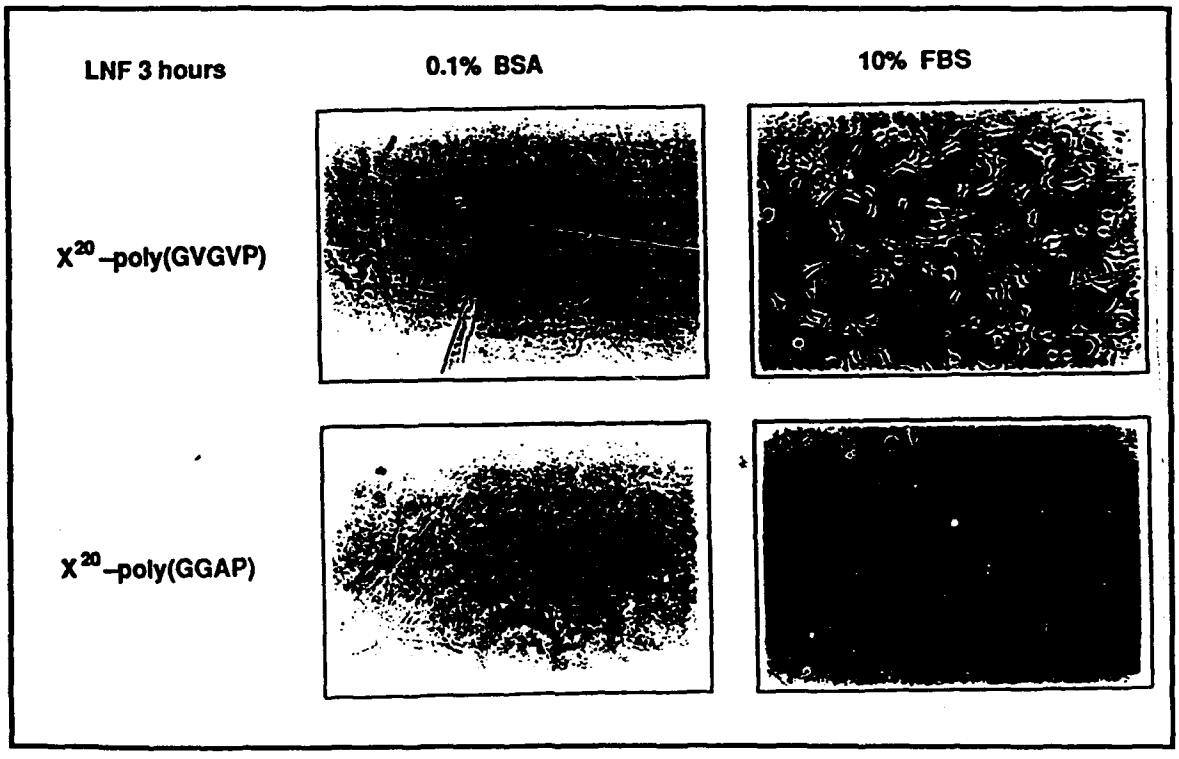

Figure 4

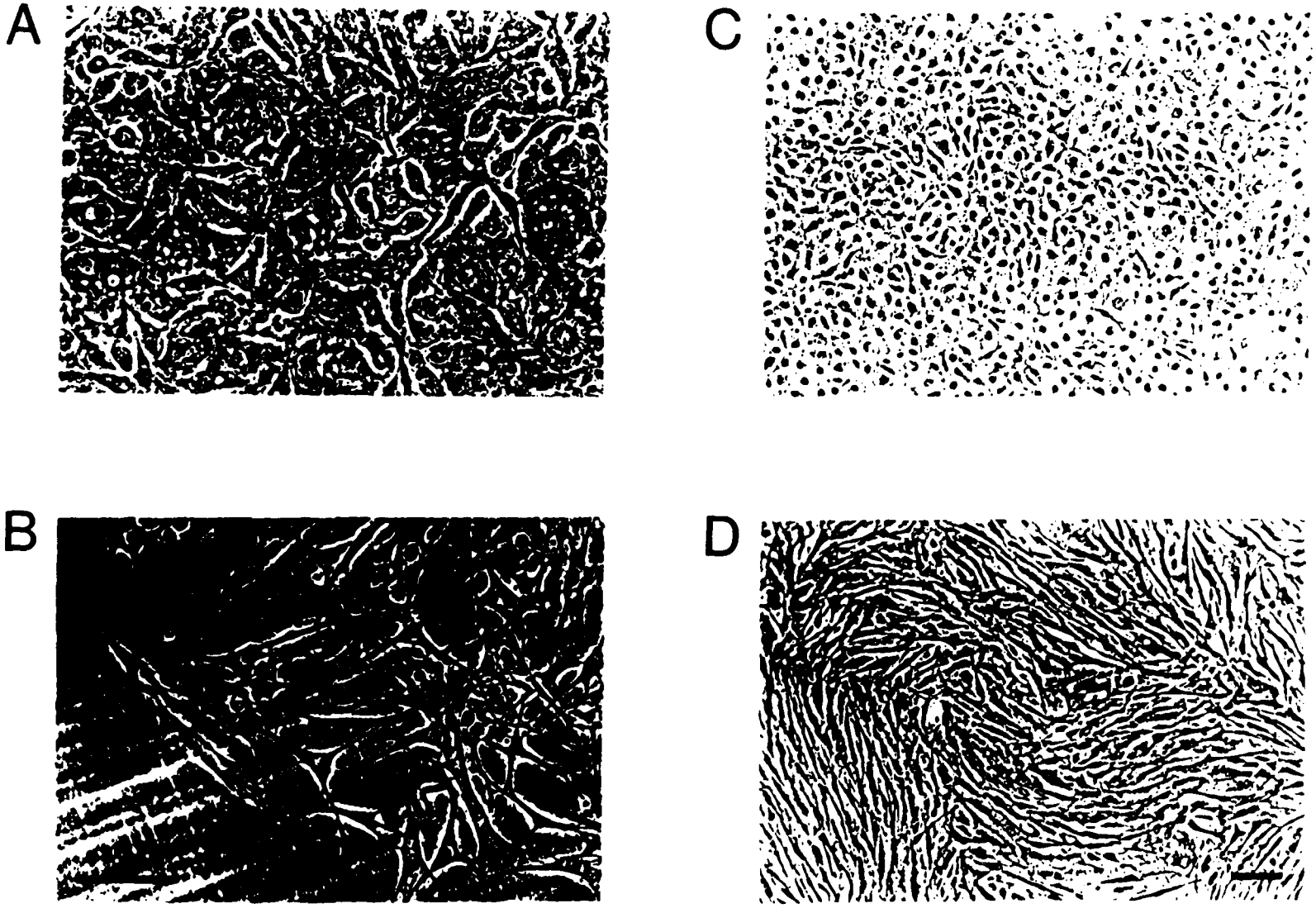

Figure 5

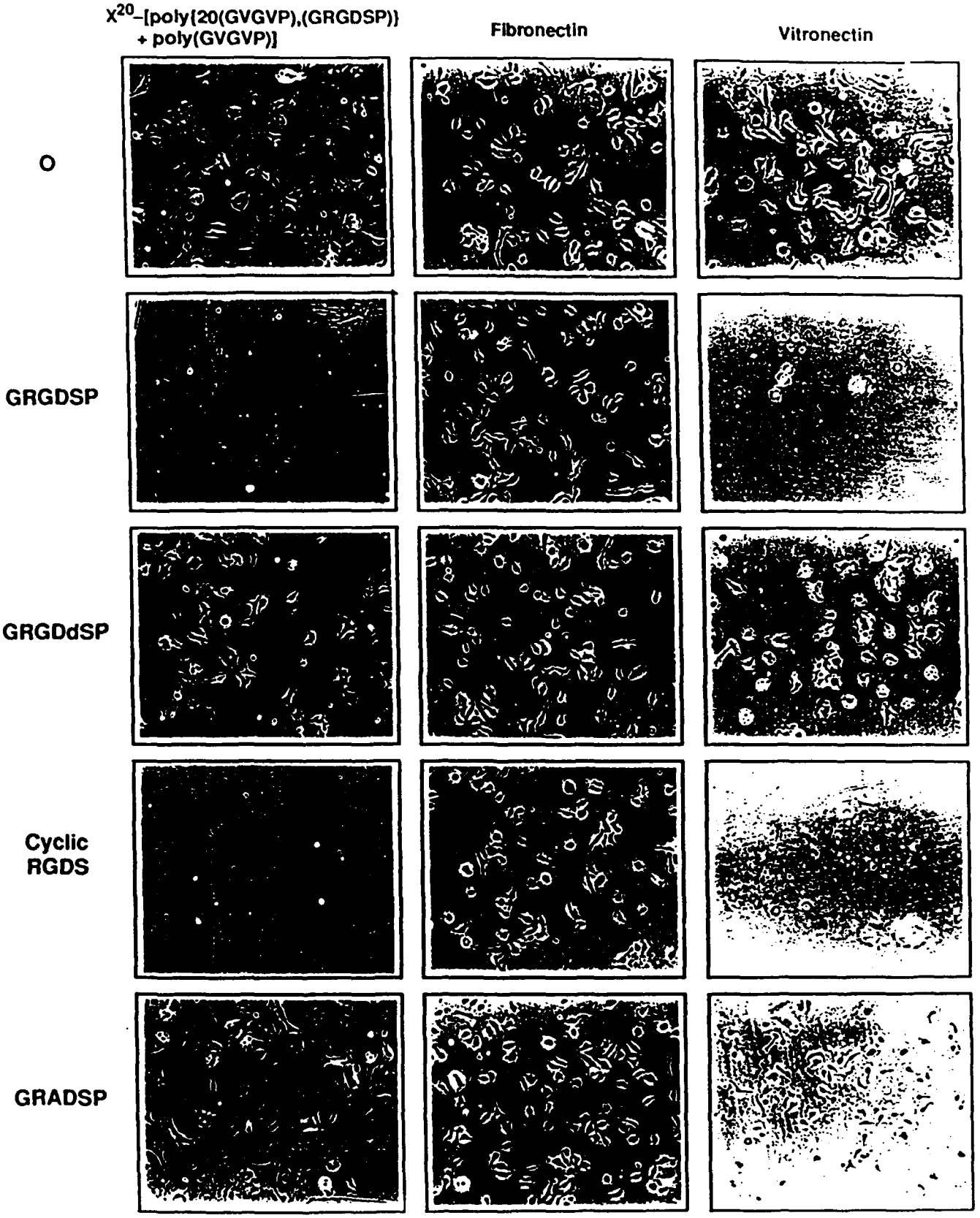
X20–[poly{20(GVGVP),(GRGDSP)} + poly(GVGVP)]
Fibronectin
Vitronectin
O
GRGDSP
GRGDdSP
Cyclic RGDS
GRADSP

Figure 6

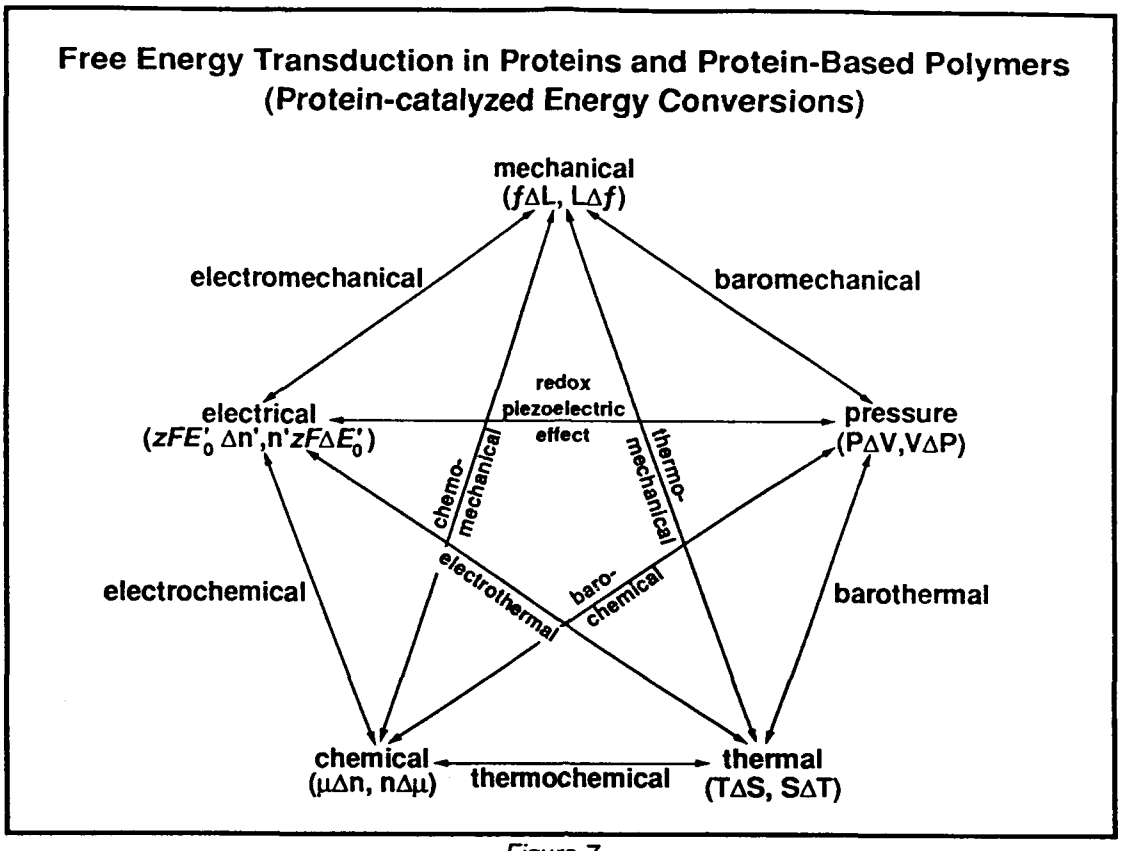

Figure 7

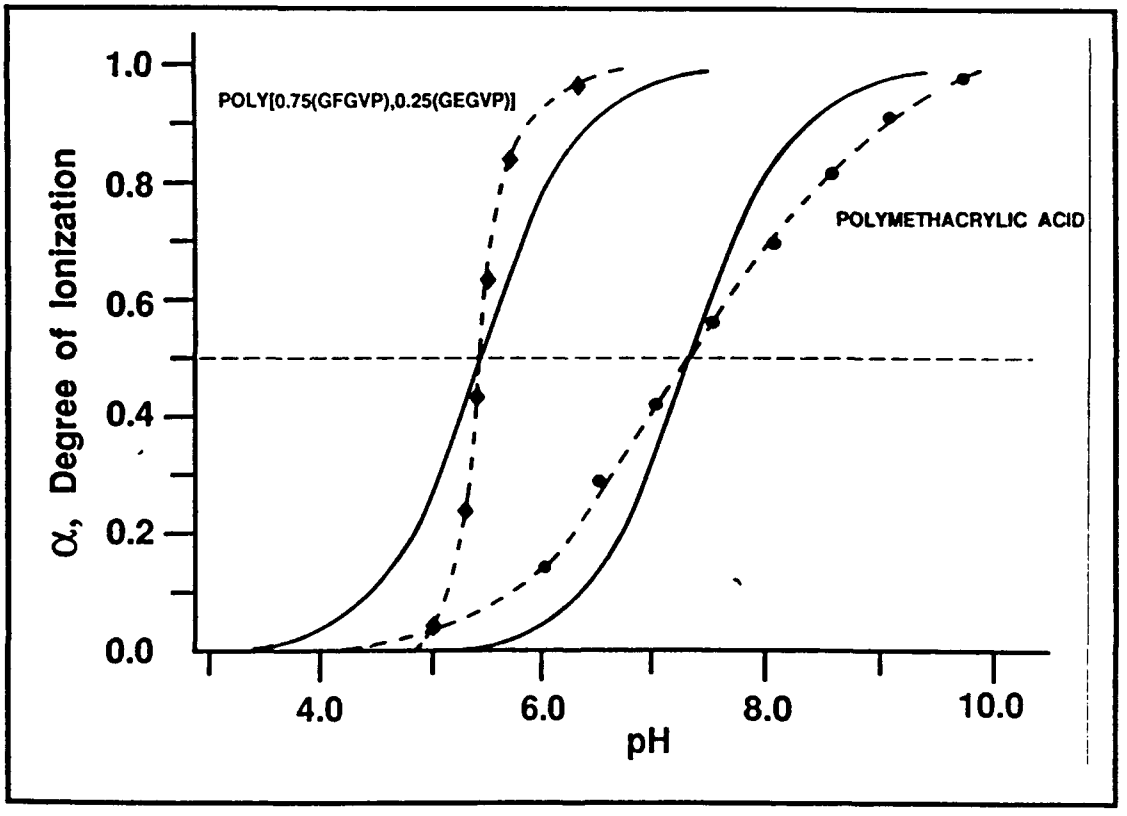

Figure 8

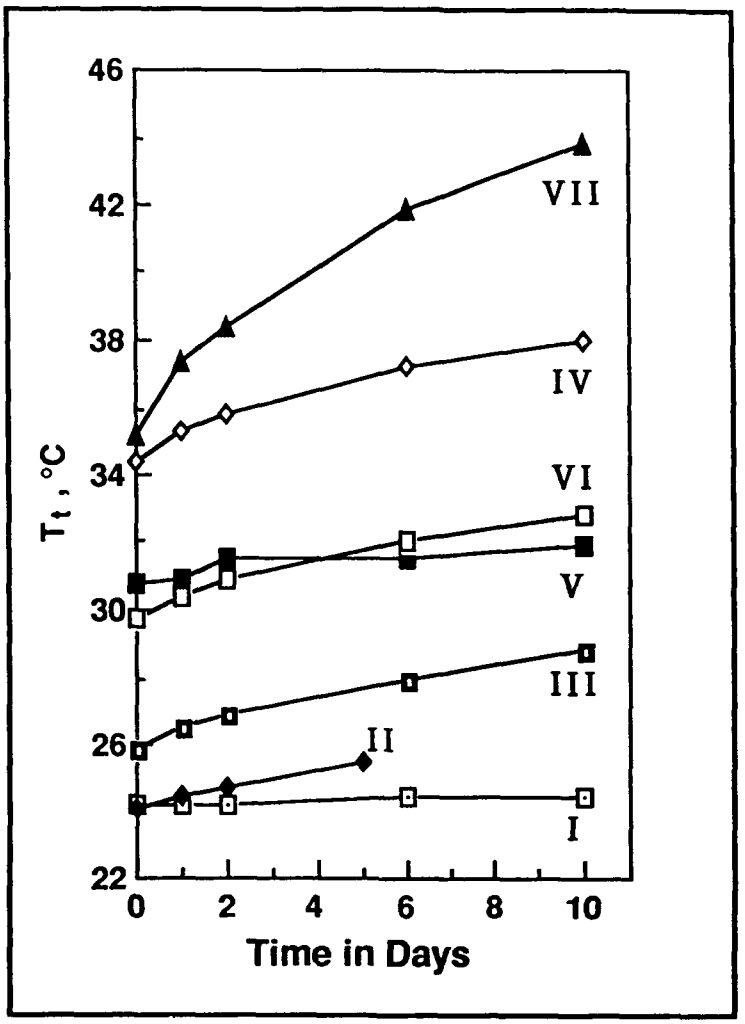

Figure 9

TABLE I

Summary of Biological Test Results for Poly(GVGVP) and its Cross-Linked Matrix X^{20}–Poly(GVGVP)

Test	Description	Test System	Results
(1) Ames (Mutagenicity)	Determine reversion rate to wild type of histidine-dependent mutants	Salmonella typhimurium	non-mutagenic
(2) Cytoxicity	Agarose overlay determine cell death and zone of lysis	L-929 mouse fibroblast	non-toxic
(3) Systemic Toxicity	Evaluate acute systemic toxicity from an I.V. or I.P. injection	Mice	non-toxic
(4) Intracutaneous Toxicity	Evaluate local dermal irritant or toxic effects by injection	Rabbit	non-toxic
(5) Muscle Implantation	Effect on living muscle tissue	Rabbit	favorable
(6) I.P. Implantation	Evaluate potential systemic toxicity	Rat	favorable
(7) Systemic Antigenicity (BPAT)	Evaluate general toxicology	Guinea Pigs	non-antigenic
(8) Sensitization (Kligman Test)	Dermal sensitization potential	Guinea Pigs	non-sensitizing
(9) Pyrogenicity	Determine febrile reaction	Rabbit	non-pyrogenic
(10) Clotting Study	Whole blood clotting times	Dog	normal clotting time
(11) Hemolysis	Level of hemolysis in the blood	Rabbit blood	non-hemolytic

*Reports from North American Science Associates (NAmSA)

PROPERTIES AND PREVENTION OF ADHESIONS APPLICATIONS OF BIOELASTIC MATERIALS

#408

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ABSTRACT

The origins, syntheses, variable composition and physical properties of bioelastic materials are discussed. The latter includes their capacity to undergo inverse temperature transitions to increased order on raising the temperature and to be designable to interconvert free energies involving the intensive variables of mechanical force, temperature, pressure, chemical potential, electrochemical potential and light.

Bioelastic materials include analogues and other chemical variations of the viscoelastic polypeptide, poly(Val-Pro-Gly-Val-Gly), and cross-linked elastomeric matrices thereof. This parent material has been shown to be remarkably biocompatible; it can be minimally modified to vary the rate of hydrolytic breakdown; it can contain enzymatically reactive sites; and it can have cell attachment sites included which promote excellent cell adhesion, spreading and growth to confluence.

One specific application is in the prevention of postoperative adhesion. There are some 30,000,000 per year surgical procedures in this country and a large portion of these would benefit if a suitable material were available for preventing adhesions. Bioelastic materials have been tested in a contaminated peritoneal model, and promising preliminary studies have been carried out in the rabbit eye model for strabismus surgery. In the peritoneal model, 90% of the 29 control animals exhibited significant adhesions; whereas, only 20% of the 29 animals using gas sterilized matrices had significant adhesions. On the basis of this data, it appears that cross-linked poly(VPGVG) is an effective physical barrier to adhesion formation in a trauma model with resulting hemorrhage and contamination.

The potential use of bioelastic materials as a pericardial substitute following the more than 400,000 open heart surgeries per year in the U.S. is under development beginning with the use of bioelastic matrices to prevent adhesions to the total artificial heart being used as a bridge to heart transplantation such that the site will be less compromised when receiving the donor heart.

BIOELASTIC MATERIALS

Origins of Bioelastic Materials

Bioelastic materials have their origins in repeating sequences of the mammalian elastic protein, elastin[1,2]. The most prominent repeating sequence occurs in bovine elastin; it can be written $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Val^4\text{-}Gly^5)_n$ where n is eleven without a single substitution. Another repeat first found in porcine elastin is $(Val^1\text{-}Pro^2\text{-}Gly^3\text{-}Gly^4)_n$ but this repeat has not been found to occur with n greater than 2 without substitution[3]. High polymers of both of these repeats, written as poly(VPGVG) and poly(VPGG) or equivalently as poly(GVGVP) and poly(GGVP), form viscoelastic phases in water and when γ-irradiation cross-linked form elastic matrices[4,5].

Compositions of Bioelastic Materials

A wide range of compositions of bioelastic materials becomes possible when substitutions are carried out in a way that does not disrupt higher order structure formation and elastic function[6,7]. For the series of primary structures that have been considered in the prevention of adhesions applications the following general structural formula may be written for the polypentapeptides as

$$\text{poly}[f_V(\text{VPGVG}),f_X(\text{VPGXG}),f_I(\text{IPGVG})] \quad [1]$$

and for the polytetrapeptides as

$$\text{poly}[f_V(\text{VPGG}),f_X(\text{XPGG})] \quad [2]$$

where the f_i are mole fractions such that in each formula the sum of f_i is equal to one and where V = Val, P = Pro, G = Gly, I = Ile and X can be any naturally occurring amino acid or a chemical modification thereof.

The compositions may be further modified to contain enzymatically reactive sites or cell attachment sites. An example of the former is poly[30(IPGVG)(RGYSLG)] where (RGYSLG), i.e., Arg-Gly-Tyr-Ser-Leu-Gly, is a specific kinase site wherein a cardiac cyclic AMP dependent kinase can phosphorylate the Ser residue and the phosphate can be removed by intestinal alkaline phosphatase[8]. An example of inclusion of a cell attachment site is poly[40(GVGVP),(GRGDSP)] where (GRGDSP), i.e., Gly-Arg-Gly-Asp-Ser-Pro, is a cell attachment sequence from fibronectin. Whereas in a standard culture medium cells do not adhere to the matrix comprised of poly(GVGVP), they do adhere, spread and grow to confluence when GRGDSP is within the elastic matrix[9].

A further modification could be the introduction of a site for proteolytic cleavage by enzymes in the milieu of interest or by enzymes doped in the matrix for the purpose of controlling rate of degradation. The matrices can be used for releasing therapeutic agents whether being employed in the prevention of adhesions or in other drug delivery contexts. Also, the chemical synthesis wherein glycolic acid residues, Glc, replaced either of the Gly residues can provide a means of controlling rate of degradation for removal and/or for release of drugs[10].

Preparation of Bioelastic Matrices

Polymers based on the repeating elastin sequences and their amino acid analogues can be synthesized microbially by using genetic engineering[11] and they can be synthesized chemically by using classical solution and/or solid phase peptide synthesis methods or by using a combination of microbial and chemical means[11,12]. For poly(GVGVP) an estimate of the cost for large scale chemical synthesis is of the order of $20.00/gram and for large scale microbial synthesis of less than $1.00/gram.

For many applications elastomeric sheets or matrices are desired. While there are many chemical and enzymatic means of achieving cross-linking to form elastic matrices, a particularly convenient method is by γ-irradiation. An effective dose is 20 Mrads with the resulting elastic matrix being designated for example as X^{20}-poly(GVGVP). One gram of poly(GVGVP) can result in a matrix 7 cm x 7 cm x 0.4 mm when contracted and 15 cm x 15 cm x 1 cm when swollen in water. Interestingly, for protein-based polymers of Formula [1] above, nuclear magnetic resonance (even using nitrogen-15 and carbon-13 enrichment)[13,14] and amino acid analyses (in preparation) before and after 20 Mrad γ-irradiation cross-linking indicate amino acid destruction to be below detectable levels.

Physical Properties of Bioelastic Materials

Inverse Temperature Transitions: Protein-based polymers of Formulae [1] and [2] as well as a number of other compositions such as poly(APGVGV) and poly(VPGFGVGAG) are

soluble in water at low enough temperatures but on raising the temperature they self-associate with clear examples of unambiguous increase in order. This increase in order on increasing the temperature is called an inverse temperature transition[15].

T_t-based Hydrophobicity Scale: On introduction of a more hydrophobic residue, e.g., Val → Ile, the temperature of the transition, T_t, is lowered and on introduction of a less hydrophobic residue, e.g., Val → Ala, the temperature of the transition, T_t, is raised. In fact, a hydrophobicity scale has been developed for all of the naturally occurring amino acid residues, their different states of ionization when relevant, chemical modifications, and biologically relevant prosthetic groups[7]. This is referred to as the T_t-based hydrophobicity scale; it provides the molecular engineer with the capacity to design materials of desired properties.

Elasticity: The elastic (Young's) modulus for X^{20}-poly(GVGVP) is about 1 x 10^6 dynes/cm^2 (10^5 Newtons/m^2) with little or no hysteresis and with extensions of up to 200% having been observed. The elastic modulus is proportional to the square of the γ-irradiation dose; for example, a doubling of the dose quadruples the elastic modulus. Depending on the composition the elastic modulus for a 20 Mrad dose can vary from 10^5 dynes/cm^2 to 10^9 dynes/cm^2. When determining the temperature, T, dependence of force, f, at fixed length, a plot of ln (f/T) versus T approximates a zero slope when above the transition temperature range such that by classical arguments X^{20}-poly(GVGVP) is a dominantly entropic (ideal) elastomer[6] as is natural elastin with the potential for the remarkable durability exhibited by natural elastin which appears to be capable of sustaining billions of demanding stretch/relaxation cycles in the aortic arch.

Thermomechanical Transduction: For those compositions such as Formulae [1] and [2] that form viscoelastic phases above the temperature, T_t, of the inverse temperature transition, they may be shaped as desired, e.g., as sheets, and γ-irradiation cross-linked to form the elastic matrices described above. These matrices exhibit reversible contraction and relaxation, i.e., de-swelling and swelling, on passing through the inverse temperature transition. On raising the temperature from below to above T_t the elastic matrices can contract and perform the mechanical work of lifting a weight. They can perform thermomechanical transduction[15,16].

The ΔT_t-Mechanism of Free Energy Transduction: Now instead of raising the temperature from below to above T_t to drive contraction, it has been shown that there are many ways to lower the value of T_t from above to below a working temperature to achieve contraction and the performance of mechanical work. This is called the ΔT_t-mechanism of free energy transduction[15,16]. Decreasing the degree of ionization of a Glu(E) residue, for example, is a dramatic means of lowering the value of T_t and addition of protons to a Glu-containing matrix has been shown isothermally to drive contraction. This is referred to as intrinsic chemo-mechanical transduction. As increasing salt (NaCl)

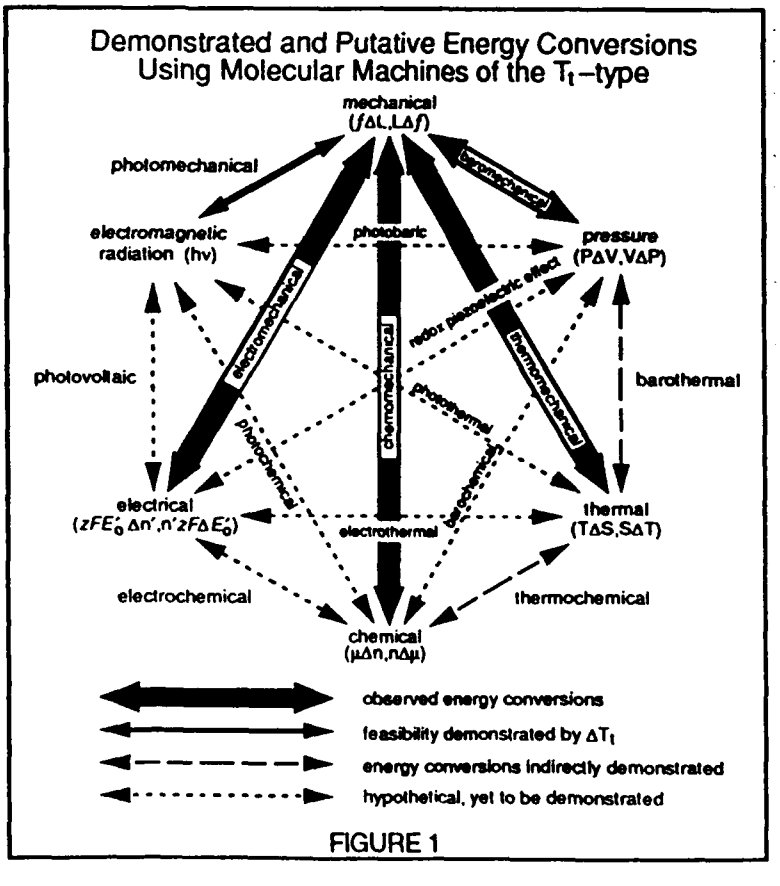

FIGURE 1

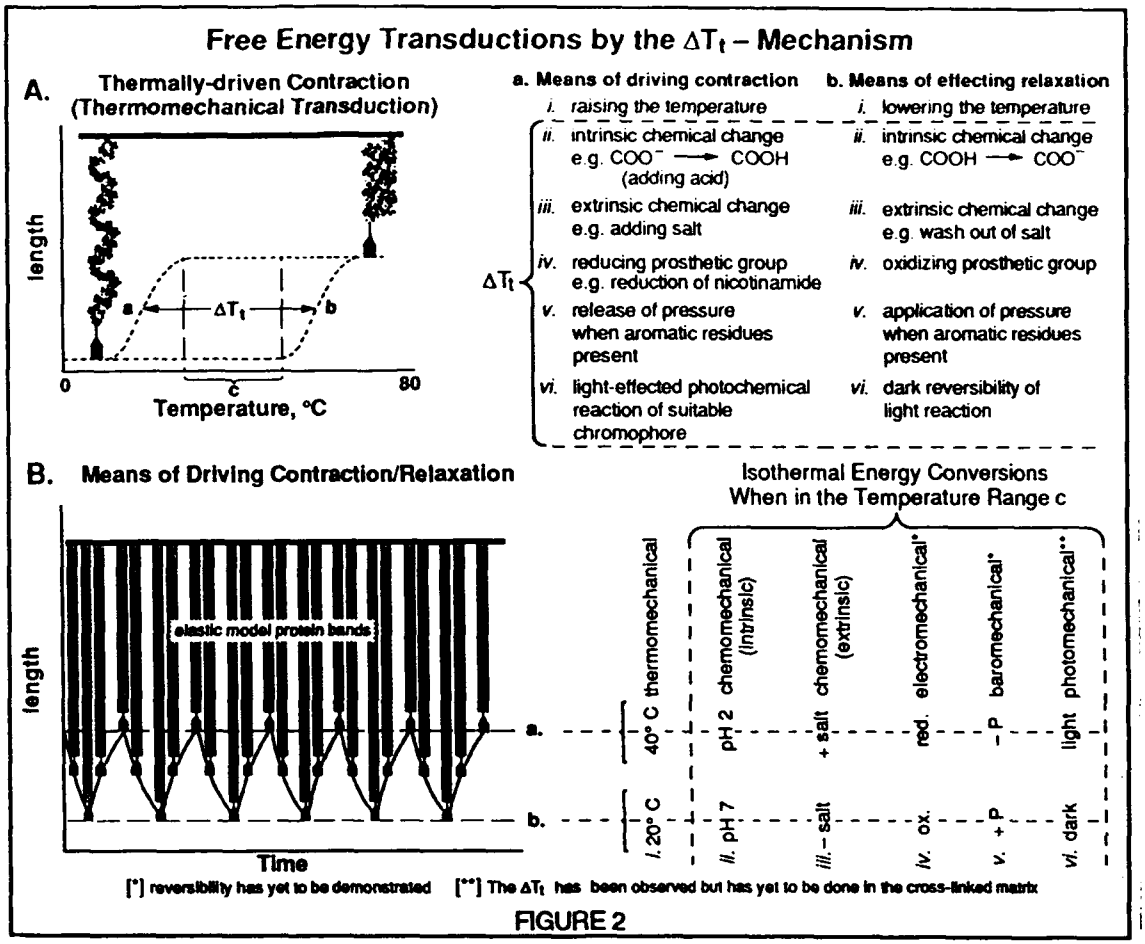

FIGURE 2

concentration lowers T_t, this too becomes a means of driving contraction referred to as extrinsic chemomechanical transduction.

Energy Conversions Possible by the ΔT_t-mechanism: In fact, the bioelastic matrices can be designed for many forms of free energy conversion involving the intensive variables of temperature, pressure, chemical potential, electrochemical potential, light and mechanical force as depicted in Figure 1. A summary of the ΔT_t-mechanism is given in Figure 2[16].

Biocompatibility of Bioelastic Materials

With the mammalian origin and nature (dynamic with a dominantly hydrophobic structure) of the bioelastic materials, it had been anticipated that these materials would be biocompatible. Following the recommendations for the set of generic biological tests required to establish biocompatibility for materials in contact with tissues, tissue fluids and blood, eleven tests were performed on poly(GVGVP) and the elastic matrix, X^{20}-poly(GVGVP). With the results following in parenthesis, these were: "(1) the Ames mutagenicity test (non-mutagenic), (2) cytotoxicity-agarose overlay (non-toxic), (3) acute systemic toxicity (non-toxic), (4) intracutaneous toxicity (non-toxic), (5) muscle implantation (favorable), (6) acute intraperitoneal toxicity (non-toxic), (7) systemic antigenicity (non-antigenic), (8) dermal sensitization—the Magnusson and Kligman maximization method (non-sensitizing), (9) pyrogenicity (non-pyrogenic), (10) Lee-White clotting study (normal clotting time), and (11) *in vitro* hemolysis test (non-hemolytic)"[17]. The result is a remarkable biocompatibility.

In addition, biocompatibility tests are underway on a member of the polytetrapeptide series, namely poly(GGAP) and X^{20}-poly(GGAP). To date the series of tests—the Ames mutagenicity test, cytotoxicity-agarose overlay, systemic toxicity, Kligman sensitization and hemolysis—also underscore good biocompatibility. Further information is available from peritoneal implants in the rat where numerous additional compositions of the Formulae [1] and [2] class of bioelastic materials all appeared to be biocompatible with X = Phe(F), Ala(A), Glu(E), and Ile(I).

Cell Attachment to Bioelastic Matrices

The bioelastic matrices—X^{20}-poly(GVGVP), X^{20}-poly(GGIP), X^{20}-poly(GGVP) and X^{20}-poly(GGAP)—do not result in cell adhesion by fibroblasts and vascular endothelial cells in appropriate cell culture media. When 10% fetal bovine serum is used in place of 0.1% bovine serum albumin, cell adhesion is observed with bovine ligamentum nuchae fibroblasts adhering better than human umbilical vein endothelial cells and with the order of decreasing cell adhesion being X^{20}-poly(GGIP) > X^{20}-poly(GGVP) > X^{20}-poly(GVGVP) but with no cell adhesion even in the presence of serum for X^{20}-poly(GGAP)[18].

On introduction of the GRGDSP cell attachment sequence[19], as in X^{20}-poly[40(GVGVP),(GRGDSP)], the bioelastic matrix presents a surface on which cells will attach and spread and grow to confluence[9]. The cells include bovine ligamentum nuchae fibroblasts, bovine aortic endothelial cells, human umbilical vein endothelial cells, and a human A375 malignant melanoma cell line[9,18,20].

Interestingly, the GRGDSP sequence as presented at the surface of this bioelastic matrix has been shown to be an attachment site for the vitronectin cell membrane receptor[20] rather than the fibronectin cell membrane receptor as might have been expected, as GRGDSP is the sequence in fibronectin that binds to the fibronectin cell membrane receptor[19]. Since blood platelets contain the fibronectin cell membrane receptor, this surface has the advantage of being very favorable for vascular endothelial cell attachment without favoring unwanted blood platelet adhesion and activation.

Another advantage of the bioelastic matrix designed for cell attachment arises because of its inherent elasticity and the capacity to vary the stiffness (the elastic modulus) of the matrix over a wide range of values from 10^5 dynes/cm^2 to 10^9 dynes/cm^2 ranging from a gelatin-like substance to a plastic-like material. Importantly, cells attached to a bioelastic matrix, as to the natural extracellular matrix, could sense deformations to which the matrix may be subjected in its role as a prosthesis, i.e., as a tissue substitute or replacement. Cells capable of sensing the tensional forces to which a tissue or a prosthesis is subjected function as mechanochemical transducers with the release of intracellular chemical signals that turn on genes for producing protein necessary for maintaining or reconstructing the extracellular matrix[21-23]. In this way a biodegradable bioelastic matrix could act as a temporary functional scaffolding which would have the potential to be remodeled into a natural tissue.

PREVENTION OF ADHESIONS APPLICATIONS

More than 30 million surgical procedures are performed annually in the U. S. with an equivalent number in Europe. In most of these adhesions, the formation of unwanted fibrous scar tissue binding tissues and organs together that should otherwise be separated, are a significant, and too often a severe, complication. An interesting example of a growing subset of these surgical procedures are the more than 400,000 open heart surgeries performed annually in the U. S. which require cardiopulmonary bypass (CPB) with the attending CPB-induced swelling of the heart which in turn commonly necessitates leaving open the pericardial sac which normally surrounds the heart. The result can be adhesion of

heart to the sternum, as well as other adhesions, with great danger of lacerating the heart on repeat sternotomy. A substantial number, approaching 20% at some centers, of the open heart procedures are reoperations with far greater risk due to the adhesions. In the abdominal cavity, post-operative and trauma-induced adhesions cause great discomfort and even intestinal blockage requiring reoperation with again increased risk in part due to adhesions obscuring the usual anatomical landmarks which guide the surgeon.

A Contaminated Peritoneal Model in the Rat[25]

Bioelastic materials have been tested in an abdominal cavity model where, as depicted in Figure 3A, the abdominal wall is scraped with a scalpel until bleeding; a loop of intestine is repeatedly punctured with a hypodermic needle until bleeding and bowel contents can be extruded; and the injured contaminated intestine is held in apposition to the injured wall by a loose loop of suture accessible without reopening the cavity. At seven days the suture loop is removed and at two weeks the abdominal cavity is reopened and examined. This results in the intestine being bound to the wall by adhesions in 100% of the cases (29 animals) with adhesions being significant in 90% of the animals[25]. Seen in Figures 4A and B for these control animals and identified by the arrows are adhesions binding loop of bowel to abdominal wall.

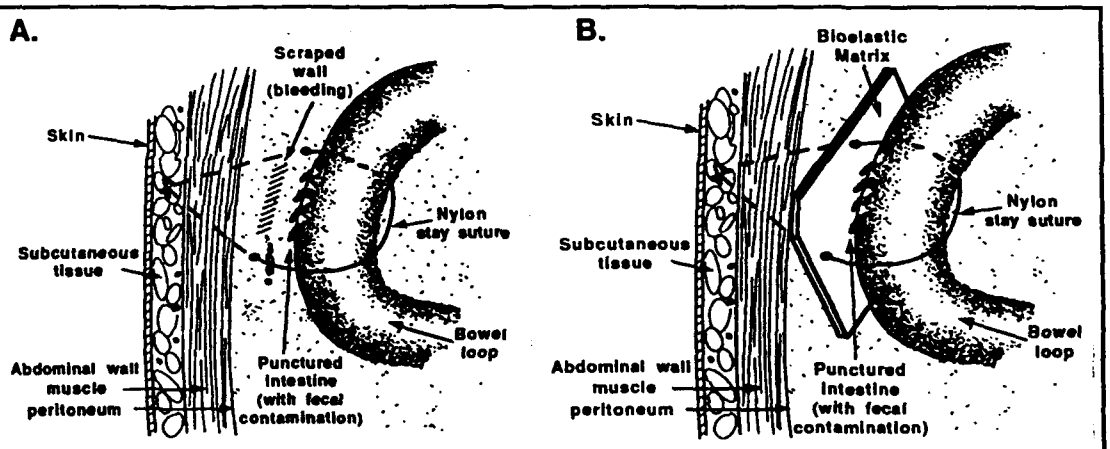

FIGURE 3

When the gas sterilized bioelastic sheet is interposed between injured wall and injured intestine as schematically shown in Figure 3B and photographed in Figure 4C, significant adhesions were prevented in 80% of 29 animals[25]. What is not apparent in the black and white print of Figure 4C is the presence of blood. In Figure 4D is the re-opened abdominal cavity showing the scarred region of the abdominal wall and the absence of any adhesions. Thus, even with the presence of blood and with frank contamination, this bioelastic matrix, X^{20}-poly(GVGVP), provided in this model an effective barrier to adhesion formation.

An instructive example is seen in Figure 4E where the vertical arrows indicate the bioelastic matrix and the horizontal arrow identifies a small loop of adhesion that has grown around the sheet of X^{20}-poly(GVGVP). The matrix is seen to have remained transparent; no fibrous coating had encapsulated the matrix in the two-week period; in fact, the matrix remains uncoated and transparent for months, seemingly ignored by the host. Through the transparent matrix it is seen that there is no sign of inflammation of the abdominal wall against which the matrix has been in contact for two weeks. Seen in Figure 4F are two bands of adhesion having grown through a break in the matrix indicated by the arrow. This occurred approximately 10% of the time, such that if this were overcome, the matrix might be expected to prevent significant adhesions some 90% of the time in this model. While other barrier materials that have been proposed for the prevention of adhesions have not

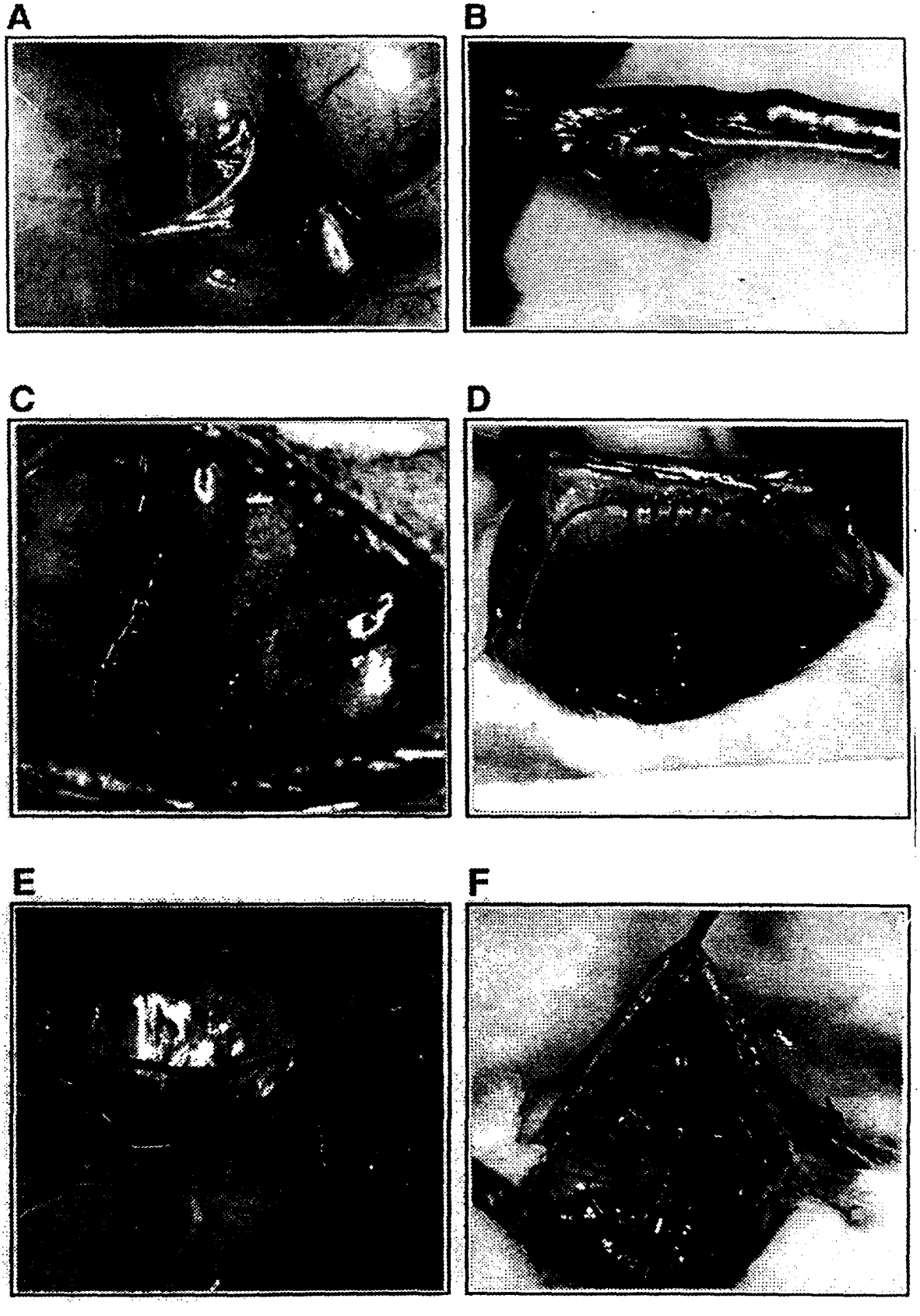

FIGURE 4

been compared in this model, this degree of efficacy has yet to be demonstrated by other materials or therapies.

These favorable findings for the X^{20}-poly(GVGVP) composition of bioelastic matrix may be obtainable by additional compositions. While there are as yet an inadequate number of animals tested for the other compositions, two were particularly promising. With four animals tested for each composition, X^{20}-poly(GGIP) was effective 100% of the time, and X^{20}-poly(GVGIP) was effective 75% of the time. Also appearing effective were X^{20}-poly[0.45(GVGVP),0.55(GAGVP)] and X^{20}-poly[0.75(GVGVP),0.25(GFGVP)]. Less effective was X^{20}-poly[0.67(GGVP),0.33(GGFP)]; with 6 animals this material was effective 50% of the time, but with the other three animals the matrix was entirely encapsulated. Exhibiting the poorest performance, but still appearing to be biocompatible, was X^{20}-poly(AVGVP) where with but three animals the material was effective 33% of the time. These additional compositions provide the opportunity for a range of physical properties; for example, they encompass the full range of elastic moduli noted above.

The serous membrane lining which covers the abdominal wall is called the parietal peritoneum, and that continuous part that is reflected over the internal organs is the visceral peritoneum. In the contaminated peritoneal model in the rat utilized above, both parietal and visceral peritoneal surfaces are injured and contaminated, and the injured sites are held in juxtaposition. This is a severe challenge to the fundamental problem of achieving repair of the serous membrane by regeneration of the mesothelial cell lining without resulting in the fibrotic response giving rise to adhesions.

Many adjunctive chemical therapies have been attempted for promoting mesothelial regeneration while limiting fibrosis resulting from surgical procedures. These include[25] heparin[26], corticosteroids[27], antihistamines[28], non-steroidal anti-inflammatory drugs[29], fibrinolytics[30], sodium carboxy methyl cellulose[31], chondroitin sulfate[32], proteolytics[33] and dextran[34,35]. Quoting from Jansen[36] in his review of "The First International Symposium for the Treatment of Post-Surgical Adhesions" held in Phoenix, Arizona September 1989, "Adjunctive therapy to promote mesothelial healing over fibrosis and formation of adhesions has a tenuous basis in the clinical practice of preventing adhesions[37-39]. Corticosteroids, antihistamines, antiprostaglandins and anticoagulants have all been used to aid healing, but properly controlled clinical studies are few and the evidence is against their use around the time of operation making a material difference to the eventual outcome."

The use of bioelastic matrices for the prevention of adhesions involves the physical barrier approach. There are many materials that have been considered as physical barriers principally as pericardial substitutes as considered further below. As recently reviewed by Gabbay[40], "These have included silicone membranes[41], polyurethane[42], fascia lata[43,44], polytetrafluoroethylene (Gore-Tex) patches[45,46], bovine and porcine pericardium xenografts (PXs) treated with glutaraldehyde[47-50], siliconized Dacron[51,52] and dura mater[53]".

Soules, et al.[54], in a comparison of the available physical barrier materials for the prevention of adhesions in the pelvic cavity, tested Gelfilm, Surgicel, Silastic, Gelfoam paste, amnion, peritoneum and omentum and concluded "The data suggest that the barrier methods actually promote the formation of adhesions...". By further designing Surgicel, specifically as a material for the prevention of adhesions, the resulting oxidized, regenerated cellulose, called Interceed (TC7), was tested in the rabbit uterine horn model[55]. Those results led to a multicenter clinical study where good surgical technique was the control with a 28% absence of adhesions and where use of Interceed and good surgical technique was the test with a 54% absence of adhesions[56]. In the Japanese multicenter clinical study for infertility and endometriosis surgery, adhesions were reduced from 76% in the controls to 41% when Interceed was used[57].

Interceed has now been approved for use in the pelvic region by the U. S. Food and Drug Administration. Even in this favorable, approved, limited anatomical use, 41% to 46% of the cases resulted in adhesion formation. In addition, Interceed is considered to promote adhesion when saturated with blood[36] and it is contra-indicated when there is

frank infection. Thus, the need for a material or therapy to prevent adhesions remains critical to improve the outcome of surgeries in general, to prevent the chronic pain and discomfort that follow abdominal surgery, to decrease the incidence of bowel obstruction following abdominal surgery, to decrease the incidence of infertility in women due to surgical procedures in the pelvic region, and to decrease risk and improve the outcome of reoperations.

Strabismus Surgery Model in the Rabbit Eye[58]

Strabismus is a disorder of the rectus muscles of the eye which prevents both eyes from simultaneously focusing on the same point, as in crossed eyes. Corrective surgery attempts to alleviate this disorder by detachment of one of the four rectus muscles that orient the eye and reattachment in order to bring the eyes into better alignment. The complication is that the repositioned muscle can adhere (become reattached due to scarring), for example, to the old insertion site, thereby defeating attempts to achieve accurate alignment.

A number of materials—silicone[59]; a polyglactin 910 mesh[60], Supramid Extra®—have been used in the form of tubes or sleeves to improve the outcome of this surgical procedure[61,62], but these efforts have now been largely discontinued. It becomes of interest therefore to determine the possible effectiveness of bioelastic materials.

In the rabbit eye model following a modification of Sondhi's method[60], the superior rectus muscle is detached at its insertion site on the sclera; a patch of sclera 3 mm x 3 mm is removed underlying the muscle; the muscle capsule overlying the scleral injury is removed, and the muscle is reattached at its original site. At one week the muscle is tightly adherent to the scleral injury site and at eight weeks histological examination demonstrated a dense fibrovascular scar. For the test animals two compositions of bioelastic materials were used, X^{20}-poly(GVGVP) with three animals and X^{20}-poly[0.75(GVGVP),0.25(GFGVP)] with two animals. Both compositions were well tolerated by the eye with no inflammation evident after the mild inflammation of the procedure subsided within a few days. In both cases adhesion of the muscle to the overlying conjunctiva and the muscle to the sclera did not occur. A glistening fibrous capsule formed around X^{20}-poly[0.75(GVGVP),0.25(GFGVP)] within two weeks whereas no capsule formed around X^{20}-poly(GVGVP) in a two-month period. Both materials ultimately extruded through the conjunctiva of the small rabbit eye. The latter material holds promise for use in strabismus surgery, particularly if the matrix can be designed to degrade within a period of a month or two in order to prevent limiting of eye movement, and possible extrusion.

Total Artificial Heart Model in the Calf (Toward an Artificial Pericardium)

Use with the Total Artificial Heart: The properties of the bioelastic materials considered in the peritoneal model appear to be appropriate for use with the total artificial heart (TAH) as a bridge to heart transplantation. When the TAH is emplaced even for a short time, adhesions form to the surface of the device. Removal of the TAH prior to placement of the donor heart requires dissection of the adhesions which presents a compromised site for the donor heart. Work is presently underway to make sheets of X^{20}-poly(GVGVP) of appropriate size which are to be utilized by the University of Utah group under the direction of D. B. Olsen in the calf model. The periods of placement are to vary from one to six months.

Toward an Artificial Pericardium: In the more than 400,000 open heart surgeries performed per year in the U. S. , the chest is opened by splitting the sternum; the pericardial sac in which the heart resides is opened and cardiopulmonary bypass (CPB) is instituted. When the procedure, which may be the emplacement of coronary artery bypass

grafts (CABG), valve replacement or correction of congenital defects, is completed, the issue of closure is addressed. It is preferred if the pericardium can be closed, but it is often necessary to leave the pericardial sac open for several reasons[45,50,63,64]. During cardiopulmonary bypass the heart can become distended and the compression due to closure can lower the performance of the heart; compression due to closure can also compress, distort or kink the aorta-coronary bypass grafts compromising their function; the pericardium can be left open to permit drainage, and shrinkage of the pericardium may have occurred following a previous operation.

There are many conditions necessitating reoperation[50,65]: "intimal hyperplasia of saphenous vein bypass grafts, graft atherosclerosis, progression of underlying coronary artery disease[65]", prosthetic valve failure, perivalvular leakage, infection on prosthetic valves and conduits, progression of coronary artery disease necessitating repeat CABG, and congenital heart disease requiring a definitive operation following a palliative surgical procedure[50]

The increased risks on reoperation are many. Perhaps most striking are the danger of rupturing the heart as the sternum is reopened due to severe adhesions between heart and sternum resulting from having left the pericardial sac open and the danger of severing an aorta-coronary graft buried within an adhesion[66]. There is increased reoperation time, excessive bleeding due to dissection of adhesions, and degeneration of pericardial substitutes that may have been tried and adhesion of the pericardial substitute to the heart[60].

Gabbay[40] has listed desirable properties for a pericardial substitute as "(1) nonadherence to the heart and easy separability upon reoperation; (2) nonadherance to the sternum upon reoperation, so that repeat sternotomy is technically no different from the original procedure; (3) capability of mechanical attributes, and maintenance of the barrier integrity of the native pericardial sac; (4) freedom from dimensional distortion or shrinkage upon prolonged implantation; (5) convenience and technical ease of handling; (6) immunologic inertia, so as not to provoke inflammatory host response; and (7) capability of acquiring fibronolytic activity similar to nature pericardial tissue".

The consideration of bioelastic materials as a pericardial substitute presents a challenge to which these new materials are well-suited. While it would be possible to discuss bioelastic matrices in terms of each of the desired properties noted above, only three aspects will be briefly noted. One is the capacity to design bioelastic matrices to have an elastic modulus in the range exhibited by the pericardial sac; the second is the demonstrated capacity in the peritoneal and eye models noted above not to adhere to tissues undergoing repair; and the third is the capacity to introduce cell attachment sequences and to provide a matrix on which those cells can function. Thus, identification and incorporation of cell attachment sequences for the mesothelial cells that line the pericardium and for the underlying mesenchymal cells could provide for a pericardial substitute that could be remodeled to form a functional pericardium with, among other properties, a fibrinolytic activity.

ACKNOWLEDGMENT

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USE OF POLYPENTAPEPTIDES OF ELASTIN TO PREVENT POSTOPERATIVE ADHESIONS: EFFICACY IN A CONTAMINATED PERITONEAL MODEL

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ABSTRACT

We investigated the use of a sheet of polypentapeptide of elastin as a physical barrier to adhesion formation in a contaminated peritoneal wound model. A total of 88 rats were studied with random assignment of animals to three study groups: Control (29), polypentapeptide steam sterilized (30), and polypentapeptide gas sterilized (29). Animals were anesthetized and a laparotomy conducted to reveal the cranial portion of the ileum. The abdominal wall muscle peritoneum was excoriated until hemorrhage was noted. In sham animals, there was no physical barrier placed between bowel loop and the abdominal wall. In two of the study groups, the polypentapeptide sheet was placed directly over the excoriated area. The intestinal loop was then loosely secured to excoriated area with 2-0 nylon (stay suture) which was tied subcutaneously in all groups. Four puncture wounds were made with a 20-gauge hypodermic needle in the bowel that was apposed to the excoriated peritoneal musculature which allowed leakage of intestinal contents and contamination. On day 7 post surgery, the animals were anesthetized and the stay suture was removed. On day 14, all animals were sacrificed and adhesions graded. The incidence of significant adhesions was 28% for barrier group versus 90% for control animals ($p<0.05$). The results of this study indicates that the polypentapeptide of elastin sheet is an effective physical barrier in this surgically induced contaminated wound model.

INTRODUCTION

Despite the advances in modern surgical techniques, postoperative intra-abdominal adhesions is a common sequela of pelvic and abdominal operations [1, 2]. Abdominal adhesions consist of fibrofatty tissue that interconnects loops of bowel or solid organs such as the liver, spleen, or intestines. It is estimated that 67% to 93% of mechanical small-bowel obstructions are caused by intraperitoneal adhesions [2, 1]. The presence of dense adhesion makes re-operation technically challenging because of the loss of intra-abdominal landmarks. Operations in this situation are often associated with increased bleeding, inadvertent enterotomy, and subsequent soilage of the operative field. Adhesions are a major cause of morbidity, including short-gut syndrome, chronic pain and female infertility [3, 4]. Many substances have been employed in the prevention of adhesions, including heparin [5], surgical barriers [6], corticosteroids [7], antihistamines [8], nonsteroidal anti-inflammatory drugs [9], fibrinolytics [10] sodium carboxymethylcellulose [11], chondroitin sulfate [12], procoagulants [9], proteolytics [13], and dextran [14]. To date, these agents have not been proven to be consistently effective, especially in the presence of inadequate hemostasis and bowel soilage.

Physical barrier and coating products have been investigated more intensely in the past several years [15, 11]. The newest

physical barrier product is Interceed® (TC7), an oxidized regenerated cellulose, which is self-adhering and absorbable [16]. When properly utilized, Interceed® (TC7) reduced the extent and severity of post-surgical adhesions in a clinical trial [16]. However, the use of Interceed® (TC7) in the presence of frank infection is contraindicated. For best results, the manufacturer recommends that complete hemostasis be established prior to the instillation of Interceed® (TC7) since the effectiveness of this product is reduced when it is saturated with blood.

This study was undertaken to evaluate the effectiveness of a polypentapeptide of elastin (polymer) as a physical barrier to adhesion in a contaminated abdominal wound in rats. These protein-based polymers of elastin were engineered to be biocompatible. The polymer is composed of a series of peptides which have been cross-linked by gamma-irradiation to form elastomeric sheets measuring approximately 2" x 2".

Preliminary studies indicated that polymer val-pro-gly-val-gly (poly(VPGVG)), which exhibits excellent biocompatibility [17], would serve as a physical barrier to adhesions in the presence of bowel soilage and hemorrhage. The emphasis of this study was to utilize the polymer in a simulated abdominal trauma model with resulting hemorrhage and contamination.

MATERIALS AND METHODS

Peptide Synthesis

The chemical synthesis and characterization of poly(GVGVP) has been extensively reported elsewhere [18, 19]. It is to be emphasized that considerable care is required to purify and characterize the intermediates that are used in the preparation of the pentamer and in the pentamer itself which is then polymerized to make the high molecular weight polymers. Small impurities, including racemization, that can occur a side reaction during the synthesis can produce significantly different properties in the final polymer.

The high polymer poly(GVGVP) is soluble in water below 25°C. On raising the temperature, aggregation occurs with settling to form the coacervate phase. The aggregation is monitored by the onset of turbidity. The temperature for onset of turbidity provides a critical assay as to the quality of the synthesis. The correct temperature for the onset of turbidity for poly(GVGVP) is 25.5°C ± 1°C. ^{13}C NMR spectra to verify the synthesis are also routinely obtained. The presence of all requisite peaks and the absence of extraneous peaks is required to verify the synthesis. This is done not only with the final product, but also with the pentamer building blocks.

Preparation of the Cross-Linked Matrix

Poly(GVGVP) is dissolved in pyrogen-free water in the concentration of 250 mg/ml. The solution is then placed in a

mold and centrifuged with the temperature maintained at 10°C below the transition. The temperature is then raised to 10°C above the transition over a period of one hour and then centrifuged for four more hours. The coacervate phase is then checked for uniformity. If it does not have any irregularities such as bubbles, it is then gamma-irradiated with a 20 Mrad dose of cobalt-60 radiation to form the cross-links which result in an insoluble matrix. The molds are then opened in a laminar flow hood using sterile conditions and placed in a tube containing sterile water. The tube is then sealed until prepared for implantation.

All of the polypentapeptide material was sterilized by either heat or gas. Heat sterilization was accomplished by autoclaving in sterile saline for 20 minutes at 120°C. The polymer was secured in gauze and a gas permeable sealed bag for ethylene oxide sterilization. Once sterilized, all polymer specimens were incubated at 37°C for 20 min. in 0.9% NaCl to allow for rehydration of the sheets prior to placement in the study animals.

Surgical Procedure

A total of 88 rats were studied with random assignment of animals to three study groups as follows: Sham-operated (n=29), polypentapeptide steam sterilized (n=30) and polypentapeptide gas

sterilized (n=29). Animals were anesthetized with isoflurane administered via nose cone. A midline abdominal incision was made through the skin and muscle tissue. The cranial portion of the ileum was located. The abdominal wall muscle peritoneum was excoriated in a small area using a #15 scalpel blade until hemorrhage was noted. At this point, the animals were divided into their respective groups. In the control animals, there was no physical barrier placed between bowel loop and the abdominal wall. In the study groups, the polypentapeptide was placed directly over the excoriated area. The intestinal loop was then loosely secured to the excoriated area with 2-0 nylon (stay suture) which was tied subcutaneously in all groups. In the case of the polypentapeptide, the stay suture was placed directly through the polypentapeptide sheet to insure proper placement. Four puncture wounds were made with a 20-gauge hypodermic needle in the bowel that was apposed to the excoriated peritoneal musculature. Intestinal contents were then milked through the puncture wounds in the bowel which contaminated the area. There was hemorrhage as a direct result of the puncture wounds. There were no attempts to acheive hemostasis. The abdominal incision was closed in a continuous pattern with 2-0 nylon and the skin with wound clips (Figure 1). Each animal was given 3cc of lactated Ringers solution subcutaneously prior to recovery from anesthesia. Animals had free access to food and water post recovery.

On day 7 post surgery, the animals were anesthetized briefly with isoflurane as before. The stay suture was surgically removed through a skin incision exposing the subcutaneously tied stay suture. The nylon suture was cut and withdrawn. The skin was closed with wound clips. At day 14, animals were euthanized with carbon dioxide via inhalation and abdominal adhesions were assessed.

Adhesions were graded according to the parameters outlined in Table 1, which are a modification of the grading system presented by Nair et al.[20]. Comparisons were made by contingency tables between treatment groups $p<0.05$.

RESULTS

Several differing amino acid sequences were evaluated (unpublished data). Their success was limited by factors such as fragility and effectiveness in preventing adhesions. The polymer (poly{VPGVG}) formulation was the most promising, as it was easy to manipulate, durable and effective.

Table 2 summarizes the results of the study. Significant adhesions were noted in 90% of the control rats studied. Since all control animals had at least one area of adhesion formation, we were satisfied with the method utilized for the creation of adhesions in this model. The incidence of significant adhesion was 28% for the polymer group versus 90% for control animals ($p<0.05$). There was no significant difference in polypentapeptide effectiveness based on sterilization techniques. Gas sterilization resulted in insignificant adhesions for 80% of the animals ($p<0.05$). Steam sterilized polymer provided protection for 56% of the animals ($p<0.05$).

Five animals had grade 2 adhesions via a defect in the polymer. These defects probably resulted from an inadvertent tear during the placement of the stay suture. In these instances, the adhesion was strictly confined to the tear in the polymer. These animals were included in the study in the grade 2 category.

The polymer was completely encapsulated in the grade 3 adhesions. Dissection through the matted bowel and fibrous connective tissue revealed an encapsulated polymer sheet. The polymer was not adhered to any of the tissues comprising the adhesion and in fact it could be easily removed with tissue forceps. The incidence of abscessation did not vary between control and polymer treated groups.

During the course of the experimental design, four animals died from bowel strangulation within 24 hours as a complication of the surgical method. These animals were not included in the study.

DISCUSSION

The pathogenesis of adhesion formation has been described [9, 21, 1, 22]. Adhesions are the most common cause of intestinal obstructions in the industrialized nations. Greater than 80% of these adhesions are the result of previous abdominal surgery [21, 23]. Post-mortem examinations reveal that at least 67% of people who have had a laparotomy developed adhesions as a direct result of this procedure. This figure rose to 93% for patients who experienced two or more procedures [1].

Much of the postoperative adhesion prevention work has been conducted utilizing models suited for the investigation of infertility. These models are designed to minimize hemorrhage and contamination. Therefore, most of the adhesion preventing substances tested to date are not effective when there is bleeding and bowel soilage. Minimal work has been done in the area of adhesions secondary to severe abdominal trauma or insult, especially when the abdominal cavity is contaminated with intestinal contents and blood. This model was designed with this principal in mind. The polypentapeptide of elastin was effective in this contaminated peritoneal wound.

Studies evaluating synthetic, absorbable barriers to adhesion formation include Poloxamer 407, Surgicel® and Interceed® (TC7). While Surgicel provided minimal protection

against adhesions, Interceed® (TC7) has been proven to reduce the incidence and severity of postoperative adhesions. However, the efficacy of Interceed® (TC7) was significantly reduced in the presence of blood and is contraindicated in the presence of frank infection [16]. While it has been demonstrated that Poloxamer 407 may have some hemostatic properties, the effectiveness in a contaminated setting has not been studied to date.

Exposure to heat results in a cis-trans isomerization change of the proline peptide in the poly-VPGVG formulation [19]. However, this alteration of the configuration did not alter the adhesion prevention effectiveness of polymer in this model.

The sheet form tested in this investigation was not self adherent and not absorbed within six months (unpublished observations). At the end of the two week period it was found floating freely within the peritoneal cavity. There was no gross or histological evidence of inflammation associated with the presence of the polymer. By these criteria, it would appear that the polymer is inert with respect to the abdominal cavity.

The polypentapeptide in sheet form has also been examined in a rabbit strabismus surgery model in which two compositions were compared: poly(VPGVG) and poly(3(VPGVG),(VPGFG)) [24]. Neither significant inflammation or significant scarring occurred with

either of the compositions, one placed surrounding the superior rectus muscle beneath the conjunctiva, where scarring always occurred in the controls. No fibrous membrane formed around poly(VPGVG) in a two-month period whereas a fibrous membrane did form around poly(3{VPGVG},{VPGFG}) within two weeks. These initial studies showed poly(VPGVG) to be promising for the prevention of adhesions in the rabbit strabismus model with the current objective being to enhance the rate of degradation. [25]

While the efficacy of the polymer sheets in this model has been addressed, the practicality has not been discussed. Part of the problem in adhesion prevention is the fact that the surgeon can not accurately predict all areas of adhesion formation. This fact will limit the success of any physical barrier sheet. Another obstacle to the use of this formulation of polymer is the that it is not self adhering. Sutures were used to hold the sheets in the proper position. The presence of suture material acting as a nidus to adhesion formation has been well documented.

We are currently investigating the possibility of obtaining liquid and foam forms of the polypentapeptide of elastin. These formulations may have more potential applications than do the polymer sheets. Liquid and foam can be evenly dispersed within the abdominal cavity and act as a bath to keep abdominal organs seperated. Like the polymer sheets, the liquid and foam formulations will also be acting as a physical barrier by

maintaining the separation of the serosal surfaces. Unlike the polymer sheets, an exact location of the adhesion formation would not be necessary, thereby making these formulations more useful in the prevention of generalized adhesions. If the serosal surfaces are kept seperated during the first 48 hours postoperatively, adhesion formation will be significantly reduced [11]. Currently we are working with increasing the viscosity of these forms in an effort to slow absorption and maintain seperation for the 48 hour period.

In conclusion, it appears that the polypentapeptide of elastin is an effective physical barrier to surgically induced adhesions in this animal model. This model also provided a source of bleeding and frank contamination to further asses the capabilities of the polymer in a trauma setting. The polymer may potentially be very useful in the prevention of postoperative intra-abdominal adhesions in contaminated wounds.

Further studies are needed to completely evaluate the possible applications of this polypentapeptide of elastin in the area of wound healing and adhesion prevention.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratroy Animals Resources, National Research Council, DHEW, Pub. No. (NIH) 78-23.

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TABLE I

Scoring Criteria for Adhesions

Grade	Description Classification
0	No Adhesions
	Insignificant Adhesions
1	Single band of adhesion composed of omental fat between omentum and abdominal wall offering no resistance to separation.
2	Involving omental fat and intestines, with a fibrous band of adhesion tissue between viscera and abdominal wall. Moderate force required for separation.Significant Adhesions
3	Abscessed adhesion involving omental fat, abdominal wall, intestines with fibrous connective tissue proliferation. Sharp dissection needed for separation.

TABLE II

Grading of Adhesions on Postmortem Examination

	% Adhesions		
Grade	Control	Gas	Steam
0	0	59(17)*	40(12)*
1	10(3)	21(6)*	16(5)*
2	62(18)	10(3)*	13(4)*
3	28(8)	10(3)*	30(9)*

*P<.05, as compared to control. (N) refers to number of rats studied

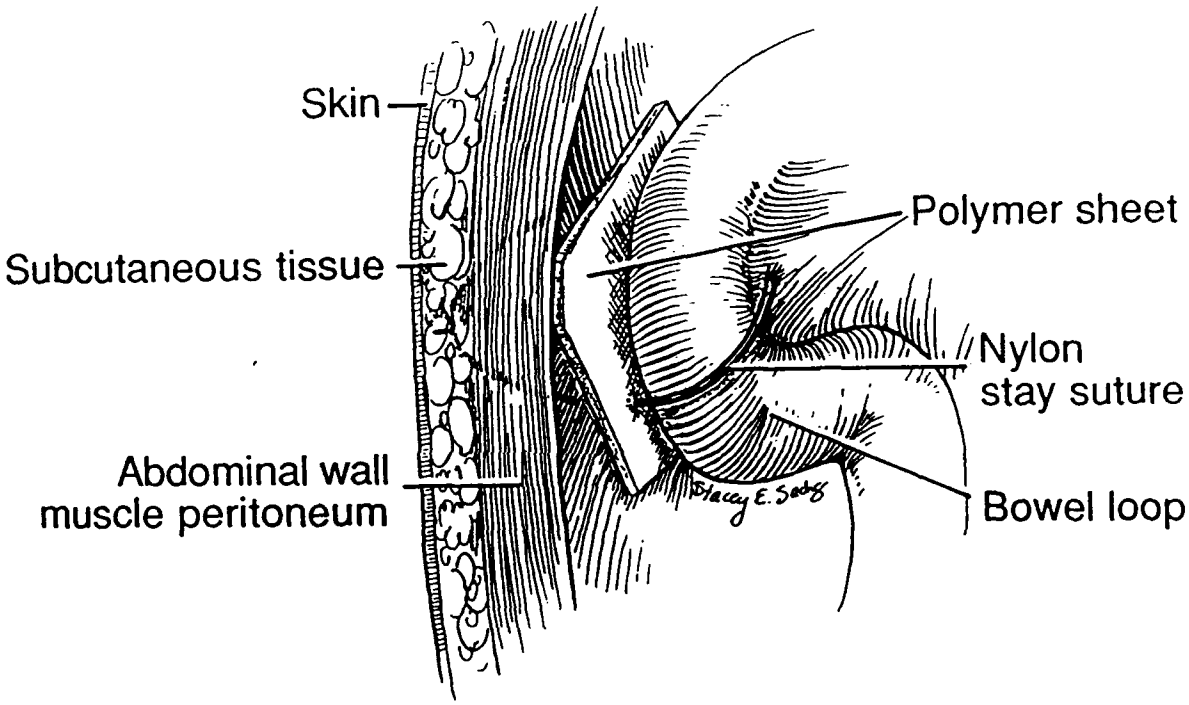

FIGURE 1 Schematic diagram of the polypentapeptide of elastin after surgical placement.

LEGEND

Figure I Schematic diagram of surgically implanted polypentapeptide of elastin.

TABLE I

Scoring Criteria for Adhesions

Grade	Description	Classification
0	No Adhesions	Insignificant Adhesions
1	Single band of adhesion composed of omental fat between omentum and abdominal wall offering no resistance to separation.	
2	Involving omental fat and intestines, with a fibrous band of adhesion tissue between viscera and abdominal wall. Moderate force required for separation.	Significant Adhesions
3	Abscessed adhesion involving omental fat, abdominal wall, intestines with fibrous connective tissue proliferation. Sharp dissection needed for separation.	