(54) Title: ELASTOMERIC POLYPEPTIDES AS VASCULAR PROSTHETIC MATERIALS

—ADVENTITIAL LAYER

INTIMAL LAYER

MEDIAL LAYER

(57) Abstract

A vascular prosthetic material having at least one layer containing an elastomeric material capable of reversibly contracting and relaxing by an inverse temperature transition. The vascular prosthetic material matches the compliance and elasticity of vessels replaced, and is biocompatible and biodegradable. The material may also have incorporated therein chemotactic peptides and cell attachment sequences.

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Description

Elastomeric Polypeptides As
Vascular Prosthetic Materials

Technical Field:

5   The present invention relates to elastomeric
polypeptides which are used as vascular prosthetic
materials.

Background Art:

         The vascular system, exclusive of the heart,
10 consists of four classes of vessels: arteries,
capillaries, veins and lymphatics. Each of these
vessels contains an elastic inner layer. See Gray's
Anatomy (The Revised American Edition based on the
Fifteenth English Edition). The elastic inner layer
15 contains fibrous elastin.
         The durability of fibrous elastin within the
vascular wall is remarkable. The half-life of this
elastin protein in the body has been estimated to be in
the range of 50 to 60 years. In the aortic arch and
20 descending thoracic aorta where there is twice as much
elastin as collagen, for example, by the age of 60
years the elastin fibers will have survived about $2x10^9$
stretch/relaxation cycles.
         This remarkable durability is due to the
25 dominantly entropic nature of the elasticity, that is,

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resistance to extension and the development of a
restoring force on extension, and not the result of the
straining of bonds on extension which could lead to an
increased probability of bond breakage.

5   Although an elastomeric fiber can be made from any
natural or synthetic material that has high elongation
and good recovery properties, at present natural rubber
and urethane polymers are used as raw materials for
elastomeric fibers. See for example U.S. patents

10   4,173,689 and 4,334,327. Although attempts have been
made to prepare elastomeric weaved polyethylene
terephthalate, polytetrafluoroethylene,
polyacrylonitriles, fabrics from polyamides,
polyesters, these woven materials have deficient

15   mechanical properties because they are not comprised of
elastomeric fibers with the appropriate compliance.

Elastomeric polyurethanes have, in general, better
mechanical properties, but are not appropriately
biocompatible and biodegradable. Moreover, they tend

20   to have elastic moduli which are too high for vascular
prostheses. Hence, vascular materials designed from
polyurethane could not be used as interim vascular
materials which are to be temporary scaffolding for the
development of new vascular tissue in the body.

25   Thus, at present, there is a need for suitable
materials which could be used to prepare vascular
prostheses. Ideally, it would be extremely desirable
if such a material could 1) match the compliance and
elasticity of vessels to be replaced, 2) have

30   incorporated therein chemotactic peptides and cell
attachment sequences for vascular endothelial and
smooth muscle cells into the vascular prosthesis, and
3) be biodegradable and biocompatible whereby natural
vessels could be regenerated, and wherein cells such as

35   smooth muscle cells when attached to an elastomeric

matrix undergoing stretch/relaxation cycles are
stimulated to produce the proteins of the vascular
wall.

Disclosure of the Invention

Accordingly, it is an object of the present
invention to provide an elastomeric material which is
advantageously used as a vascular prosthetic material.

It is, more particularly, an object of this
invention to provide elastomeric polypeptides which are
advantageously so used, and which match the compliance
of small vessels to be replaced.

Further, it is an object of this invention to
provide elastomeric polypeptides which can have
incorporated therein chemotactic peptides and cell
attachment sequences for vascular endothelial and
smooth muscle cells.

Moreover, it is an object of the present invention
to provide elastomeric polypeptides which are
biocompatible and biodegradable.

It is also an object of this invention to provide
a vascular prosthetic material which may have several
specialized layers thereto, each performing a separate
function.

In accordance with the present invention, a
vascular prosthesis is provided which entails a vessel
having at least one layer in the vessel wall containing
an elastomeric material, capable of reversibly
contracting and relaxing by an inverse temperature
transition.

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Brief Description of the Drawing

FIGURE 1 illustrates the elastic modulus (Young's Modulus) at low extensions for the cross-linked $(VPGVG)_n$ matrix as a function of γ-irradiation cross-linking dose.

FIGURE 2 illustrates stress-strain curves obtained for a band of 20 Mrad cross-linked $(VPGVG)_n$ or $(X^{20}\text{-PPP})$ for a sequence of cycles at 10% increments up to 90, which evidences the low degree of hysteresis for the intial extensions.

FIGURE 3 illustrates stress-strain curves obtained for $X^{20}\text{-}(PHP:PPP, 1:6)$ taken at larger percentage increments, which evidences the extent of hysteresis and sample rupture on approaching 200% extension.

FIGURE 4A illustrates one continuous stress-strain curve up to 320% extension for a dog femoral artery: previously unstressed.

FIGURE 4B illustrates a series of cycles of 20% extension increments for a dog femoral artery: previously unstressed.

FIGURE 4C illustrates a stress-strain curve following a single extension to about 120% for a dog femoral artery: previously stressed.

FIGURE 5 illustrates the stress at 30% extension for the internal mammary artery of dog.

FIGURE 6 illustrates the synthesis of a section of synthetic elastomeric tube: A) illustrates a suitable tube in which PPP and PHP are dissolved in water at room temperature, B) illustrates a pestle which is inserted into the formed viscoelastic phase, and which is removed upon cross-linking, and C) illustrates the formed elastomeric tube.

FIGURE 7 illustrates stress-strain curves of the
composite PHP/PPP matrix, which are
temperature dependent.
FIGURE 8A illustrates the temperature dependence of
matrix length with the apparent temperature
elicited contraction.
FIGURE 8B illustrates the elastic modulus as a
function of temperature.
FIGURE 9 illustrates a schematic drawing of an
elastomeric vessel containing a layered wall.

Best Mode For Carrying Out the Invention

In accordance with the present invention, vascular
prostheses are provided which contain elastomeric
polypeptides having many advantageous properties. In
the most basic form, an elastomeric vessel may be
prepared which contains a single-layer wall. However,
the elastomeric vessel may be constructed to have a
wall with at least three layers, or even more.

In perhaps the most fundamental form, the
elastomeric prostheses of the present invention
consists of an elastomeric tube or vessel containing
predominantly repeating units of elastin.pentapeptide
(VPGVG), with the remainder being the repeating
hexapeptide (VAPGVG). Thus, the formula of the bulk
matrix may be generally written as $[(VPGVG)_m$-
$(VAPGVG)_n]_\ell$, wherein m may have a value of about 3 to
6, and n has a value of about 10 to 15. ℓ has a value
such that upon γ-irradiation the bulk matrix will have
an appropriate elastic modulus. Generally, a molecular
weight in the range of 10,000-50,000 daltons will be
adequate as a minimal molecular weight.

The elastomeric prostheses may be constructed so
as to have one or more layers, each layer having a
specialized function. For example, in accordance with

the present invention an elastomeric vessel may be
designed to contain one or more layers having cell
attachment sequences for endothelial cells or smooth
muscle cells. Alternatively or additionally, a layer
may be designed for attachment to surrounding
connective tissue.

Further, in accordance with the present invention,
elastomeric prostheses are provided which contain
several layers each with a particular and different
function. For example, a three-layered elastomeric
vessel may be constructed, the vessel wall of which
contains 1) an intimal-inner layer specialized for cell
attachment of endothelial cells, 2) a medial-middle
layer specialized for cell attachment of smooth muscle
cells, and 3) an adventitial-outer layer specialized
for attachment to surrounding connective tissue. This
aspect of the present invention is illustrated in
FIGURE 9.

A. THE ELASTOMERIC MATERIALS

The basic elastomeric polypeptide utilized in the
construction of the present prostheses is the elastin
polypentapeptide $\{VPGVG\}_n$ or PPP. However other
peptide sequences may be added to this basic
composition, such as $\{VPGG\}_n$ or the polytetrapeptide
sequence PTP and $\{VAPGVG\}_n$ or the polyhexapaptide
PHP. A more complete description of these materials
and the synthesis thereof will now be described.
Additionally, the present inventor has conducted
previous research with elastin polypeptides, the
description of which is, in part, described in U.S.
patents 4,187,852; 4,474,851; 4,500,700; 4,589,882;
4,605,413 and copending applications S.N. 853,212 and
062,557 all of which are incorporated herein by
reference.

Elastin is comprised of a single protein. The
sequence of elastin can be described as a serial
alignment of alanine-rich, lysine-containing cross-
linking sequences alternating with glycine-rich
5   hydrophobic sequences. With the entire bovine sequence
known, the most striking hydrophobic sequences, both
from the standpoint of length and of composition, are
one that contains a polypentapeptide (PPP) and one that
contains a polyhexapeptide (PHP). Elastin also
10   contains a repeating polytetrapeptide (PTP). As a
result of work conducted by the present inventor, the
polypentapeptide of elastin when cross-linked has been
found to be elastomeric and the polyhexapeptide thereof
has been found to be non-elastomeric and appears to
15   provide a means for aligning and interlocking the
chains during elastogenesis. From the present work, it
has now also been found that the elastin
polypentapeptide and polytetrapeptide are both
conformation-based elastomers that develop entropic
20   elasticity and strength on undergoing an inverse
temperature transition to form a regular ß-turn
containing dynamic structure.

A typical biological elastic fiber is comprised of
a large elastin core covered with a fine surface layer
25   of microfibrillar protein. Elastin is formed upon
cross-linking of the lysine residues of tropoelastin.
The repeating elastin pentapeptide has the formula
$(VPGVG)_n$, while the repeating hexapeptide has the
formula $(VAPGVG)_n$, where n varies depending upon the
30   species. The repeating polytetrapeptide unit has the
formula $(VPGG)_n$. These sequences, as well as all
others recited herein, utilize either the standard one-
letter or three-letter abbreviation for the constituent
amino acids.

35   It has been found that these polypeptides are

soluble in water below 25°C, but on raising the
temperature they associate in the polypentapeptide
(PPP) and polytetrapeptide (PTP) cases, reversibly to
form a viscoelastic phase, and in the polyhexapeptide
5   (PHP) case, irreversibly to form a precipitate. On
cross-linking, the former (PPP) and (PTP) have been
found to be elastomers.

        In part, the present material contains PPP, which
exhibits aggregation and forms a water-containing
10   viscoelastic phase, which upon cross-linking by
γ-irradiation forms an elastomer. By contrast, PHP
forms a granular precipitate, which is not
elastomeric. In fact, it has been found that for
potential elastomers, such aggregation is reversible,
15   whereas for non-elastomeric samples, such as PHP,
temperature-driven aggregation is irreversible in water
and redissolution usually requires the addition of
trifluoroethanol to the aggregate.

        For purposes of clarification, it is noted that
20   the reversible temperature elicited aggregation, which
gives rise upon standing to a dense viscoelastic phase,
is called coacervation. The viscoelastic phase is
called the coacervate, and the solution above the
coacervate is referred to as the equilibrium solution.

25   Most importantly, however, cross-linked PPP, PTP
and analogs thereof at fixed length exhibit elastomeric
force development at different temperatures spanning a
range of up to about 75°C depending upon several
controllable variables. Moreover, these cross-linked
30   elastomers develop near maximum elastomeric force over
a relatively narrow temperature range. Thus, by
synthesizing bioelastomeric materials having varying
molar amounts of the constituent pentamers and
tetramers together with such units modified by
35   hexameric repeating units, and by choosing a particular

solvent to support the initial viscoelastic phase, it
is possible to rigorously control the temperature at
which the obtained bioelastomer develops elastomeric
force.

5   In general, the process of raising the temperature
to form the above elastomeric state is an inverse
temperature transition resulting in the development of
a regular non-random structure, unlike typical rubbers,
which utilizes, as a characteristic component,
10   hydrophobic intramolecular interactions. The regular
structure is proposed to be a β-spiral, a loose water-
containing helical structure with β-turns as spacers
between turns of the helix which provides hydrophobic
contacts between helical turns and has suspended
15   peptide segments. These peptide segments are free to
undergo large amplitude, low frequency rocking motions
called librations. Consequently, a new mechanism of
elasticity has now been developed called the
librational entropy mechanism of elasticity.

20   The elastomeric force of these various
bioelastomers develops as the regular structure thereof
develops. Further, a loss of regular structure by high
temperature denaturation, results in loss of
elastomeric force. Interestingly, this situation is
just the reverse of that for the random-chain-network
25   theory of elasticity; in the present case the more
nearly random the polypentapeptide, the less the
elastomeric force, and the more developed the
dynamic β-turn containing structure, the greater the
elastomeric force.

30   In the broadest sense, a new entropy-based
mechanism of elasticity is presented for use herein.
The mechanism therefor appears to be derived from a new
class of polypeptide conformations called β-spirals·
wherein β-turns recur with regularity in a loose water-
35

containing helix. The β-spiral is the result of
intramolecular interturn hydrophobic interactions which
form on raising the temperature in water. In the β-
spiral of the elastomeric polypentapeptide of elastin,
$(Val^1-Pro^2-Gly^3-Val^4-Gly^5)_n$, the type II Pro^2-Gly^3 β-
turns function as spacers, with hydrophobic contacts,
between the turns of the helix, which results in the
segments of $Val^4-Gly^5-Val^1$ being suspended. Being
essentially surrounded by water, the peptide moieties
of the suspended segments are free to undergo large
rocking motions referred to as librations which become
damped on stretching. The decrease in amplitude of
librations, and possibly an increase in their frequency
of motion, on stretching constitutes a decrease in
entropy and it appears that the decrease in free energy
due to the increase in entropy on returning to the
relaxed state is the driving force for elastomeric
retraction.

Upon raising the temperature of the polypeptide-
solvent system, such as PPP-water, for example, the
hydrophobic side chains such as those of Pro and Val
when dispersed in water are surrounded by water having
a clathrate-like structure, that is, by water that is
more ordered than normal bulk water. Upon raising the
temperature, an amount of this more ordered clathrate-
like water surrounding the hydrophobic groups becomes
less ordered bulk water as the hydrophobic chains
associate to form a more ordered polypeptide. It
appears that it is the optimization of intramolecular
hydrophobic contacts that assists the polypeptide in
wrapping up into a loose helix. Adherence to the
Second Law of Thermodynamics appears to be maintained
by the requirement that the decrease in entropy of the
polypeptide portion of the system be less than the
increase in entropy of the water in system. Since ΔG=

0 at the temperature midpoint (T_{mp}) of a structural
transition between a pair of states, then $T_{mp} = \Delta H/\Delta S$.

If the entropy change, ΔS, derives from the
hydrophobicity of the repeating unit, as it would in
the clathrate-like water mechanism, than an increase in
the hydrophobicity of the repeating unit can be used to
explain the decrease in T_{mp}, the midpoint of the
inverse temperature transition. In fact, a decrease in
the hydrophobicity of the repeating unit results in an
increased T_{mp}. Conversely, an increase in the
hydrophobicity of the repeating units results in a
decrease in T_{mp}.

The above principle is demonstrated by
substituting the more hydrophobic isoleucine (Ile) for
valine (Val) in the elastin polypentapeptide, (Ile^1-
Pro^2-Gly^3-Val^4-$\text{Gly}^5)_n$, i.e., Ile^1-PPP, to produce a
substituted polypentapeptide which has properties
similar to PPP, except that the described transition
occurs at a lower temperature.

For purposes of clarity, it is noted that for the
above numbered sequence and all sequences hereafter,
the superscript numbering system is a sequence
numbering based upon the dominant secondary structural
feature of these repeating sequences which is the type
II Pro^2-Gly^3 β-turn, a ten atom hydrogen bonded ring
involving the C=O of residue 1 and the NH of residue 4.

The present elastomeric materials also extend to
the polytetrapeptide of elastin. It is recalled that
this repeating unit has the formula (Val^1-Pro^2-Gly^3-
$\text{Gly}^4)_n$, which also forms a β-spiral similar to PPP.
However, the temperature of aggregation for PTP occurs
at a higher temperature than for PPP. In essence, for
both the polypentapeptide and polytetrapeptide
repeating units of elastin, it has been found that the
temperature of the transition for the development of

elastomeric force is inversely proportional to the
hydrophobicity of the repeating unit. Hence, two
important principles have been elucidated. First,
elastomeric force development at fixed length or the
5   shortening at fixed force occurs due to an inverse
temperature transition resulting in increased
polypeptide order by raising the temperature.
Secondly, the temperature of this transition for the
development of elastomeric force is inversely
10   proportional to the hydrophobicity of the repeating
unit in the bioelastomer.

        Analogs of both the elastin polypentapeptide PPP
and the polytetrapeptide (PTP) and combinations thereof
are contemplated for use in the present invention. For
15   example, it has been found that the temperature of
transition for Ile^1-PPP shifts to a lower temperature
by an amount calculable from the increase in
hydrophobicity relative to PPP using hydrophobicity.
Thus, by carefully choosing a new analog with a
20   different repeating unit hydrophobicity, the transition
temperature for the development of elastomeric force
can be predictably shifted to a different
temperature. In fact, by judiciously selecting various
repeating units and combinations thereof, along with
25   various solvent mixtures it is now possible to select a
transition temperature from within a range of up to
about 75°C, from about -25°C to about +50°C.

        Additionally, the elastin polyhexapeptide (PHP)
may also be incorporated in various amounts in the
30   elastomeric materials of the present invention as will
be discussed below.

        The most striking repeating sequence of bovine and
porcine elastin is the polypentapeptide Val^1-Pro^2-Gly^3-
Val^4-$Gly^5)_n$, wherein, for example, n is 11 for cows and
35   pigs. The polypentapeptide is soluble in water at all

proportions below 25°C. On raising the temperature
above 25°C, aggregation occurs and the aggregate
settles to form a dense viscoelastic phase, called a
coacervate that at 40°C is about 38% peptide and 62%

5   water by weight. The process of PPP coacervation, as
noted, is entirely reversible. Moreover, on
crosslinking, the PPP coacervate is found to be
elastomeric. The coacervate concentration of PPP as
well as the elastomeric γ-irradiation cross-linked PPP

10   coacervate undergo an inverse temperature transition,
which commences at 25°C and which reaches completion
near 37°C. Over the same temperature range, the
elastomeric force of the cross-linked PPP coacervate at
fixed length increases dramatically or the length at

15   fixed force shortens dramatically on raising the
temperature from near zero at 20°C to full force or
full contraction near 40°C. Above 40°C, the
elastomeric force or length divided by the temperature
(°K) becomes quite constant.

20   This indicates that the cross-linked PPP is a
dominantly entropic elastomer. That is, the entropic
component of the elastomeric force depends upon the
decrease in numbers of low energy states accessible to
the polymer on extension, whereas a large internal

25   energy component of elastomeric force would result from
stressing of bonds which would increase the probability
of rupture of the elastomer.

             Thus, in part, the materials of the present
invention are of a nature such that it is possible to

30   change the temperature of transition by modifying the
PPP. In particular, it has been found that by
increasing the hydrophobicity of the PPP repeating
unit, the viscoelastic phase transition occurs at lower
temperatures, while by decreasing the hydrophobicity of

35   the repeating units, this transition occurs at higher

temperatures. Of course, when modifying the
hydrophobicity, it is necessary to do so in a way such
that elasticity is retained.

For example, modifications of the repeating
pentamers have been made which destroy the molecular
structure required for elasticity, such as the Ala^1 and
Ala^5 analogs. The Ala^1 and Ala^5 analogs, the former
decreasing and the latter increasing pentamer
hydrophobicity, result in the formation of granular
precipitates on raising the temperature of aqueous
solutions rather than forming viscoelastic coacervates
and γ-irradiation cross-linking of the Ala^5-PPP
precipitate results in a hard material that simply
breaks upon stretching. In accordance with the present
discovery, it is believed that these analogs fail to
produce elastomeric polymers for different but
consistent reasons. First, the Ala^1 analog does not
appear to allow for important Val^1 γCH_3...$Pro^2 \delta CH_2$
intramolecular hydrophobic contacts required to form a
viscoelastic coacervate. The Ala^5 analog appears to
interfere with librational motions in the Val^4-Gly^5-
Val^1 suspended segment of the proposed PPP molecular
structure. As noted, the librations are central to the
proposed librational entropy mechanism of elasticity.

By contrast, the hydrophobicity of the repeating
pentamer can be easily increased by introducing a-CH_2-
moiety, for example, in residue 1 while maintaining β-
branching, that is, to utilize the Ile^1 analog of PPP,
i.e., $(Ile^1$-Pro^2-Gly^3-Val^4-$GLy^5)_n$. With a greater than
50,000 molecular weight, Ile^1-PPP reversibly forms a
viscoelastic coacervate with the onset of coacervation
being at 8°C rather than 24°C as for unsubstituted
PPP. It appears from circular dichroism data that
Ile^1-PPP and PPP have identical conformations both
before and after the transitions and that the

transition to increased intramolecular order on
increasing the temperature is also shifted by 15°C or
more to lower temperatures. Further, the dramatic
increase in elastomeric force on raising the
temperature of the γ-irradiation cross-linked
coacervate is similarly shifted to a lower temperature
for the Ile^1-PPP analog. Thus, with this analog, a
coupling of temperature dependent elastomeric force
development and molecular structure is demonstrated.
This, of course, means that it is now possible to
rationally design polypeptide elastomers that undergo
transitions at different temperatures and that would
function as entropic elastomers in different
temperature ranges.

    As noted above, by increasing the hydrophobicity
of PPP, such as by substituting Ile^1 for Val^1 in the
pentameric sequence of $-(\text{VPGVG})_n$ to form $-(\text{IPGVG})_n$, it
is now possible to accomplish at least two distinct
objectives.

    First, it is now possible to prepare, for example,
the "homopolymeric" polypentapeptide of $-(\text{IPGVG})-_n$
i.e., Ile^1-PPP, which, as noted dissolves in water at
4°C, and upon raising the temperature to 8°C, exhibits
aggregation. After cross-linking the coacervate by γ-
irradiation, it is observed for a fixed length that
essentially full elastomeric force is exhibited at
about 25°C for the cross-linked Ile^1-PPP as opposed to
the 40°C temperature required for the unsubstituted
PPP. Thus, the temperature of the ordering transition
for Ile^1-PPP occurs at a temperature approximately 15°C
lower than for PPP.

    Secondly, it is now also possible to prepare mixed
"copolymers", for example, of the polypentapeptides
$-\text{X}^1-(\text{IPGVG})_n-\text{Y}^1-$ and $-\text{X}^2-(\text{VPGVG}-)_n-\text{Y}^2-$ which exhibit
variable and controllable transition temperatures which

are in between the separate transition temperatures of
PPP and Ile^1-PPP. Further, a great degree of control
is possible inasmuch as the transition temperature
obtained is proportional to the molar ratios of the
5   respective pentapeptides incorporated therein.

        Although Ile^1-PPP is an excellent example of an
increased hydrophobicity PPP analog, any PPP analog,
which alters the hydrophobicity of the repeating
pentameric unit, while retaining the elasticity of the
10 polypeptide, and without interfering with either the
formation of the viscoelastic coacervate or the
librational motion is characteristic of the materials
to be utilized as the vascular prosthetic material.

        For example, in addition to repeating unit
15 sequences of $\{IPGVG\}_n$, using Ile^1, it is also possible
to effect a variety of other substitutions. In
general, a pentapeptide repeating unit of the formula:

$$-(R_1PR_2R_3G)_n-$$
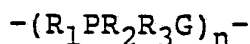
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is also capable of being used in the materials of the
present invention, wherein R_1 is selected from the
group consisting of Phe, Leu, Ile, and Val; R_2 is
selected from the group consisting of Ala and Gly; R_3
25 is selected from the group consisting of Phe, Leu, Ile,
and Val; and n is an integer from 1 to 5,000; and P is
L-proline and G is glycine.

        Notably, the above substitutions modify the
hydrophobicity of the repeating unit so as to attenuate
30 the transition temperature for near maximum elastomeric
force development, of course, without destroying the
elasticity of the bioelastomer.

        In the above formula, it is noted that the amino
acid Leu is, of course, leucine and Phe is
35 phenylalanine. The three-lettered abbreviations used

in the present specification are the standard
abbreviations for amino acids. R_1, R_2 and R_3
correspond to positions 1, 3 and 4 in the numbered
sequence as described herein.

5   As noted previously, the materials of the present
invention include not only PPP analogs, such as Ile^1-
PPP, Phe^1-PPP or Ala^3-PPP but all PPP analogs, and
bioelastomers containing the same, which have
transition temperatures, and, hence, temperatures of

10   near maximum elastomeric force development, which are
different from PPP; while retaining elasticity. Given,
the present disclosure, one skilled in the art could
clearly ascertain additional PPP analogs, and
bioelastomers incorporating the same which meet the

15   above criteria.
       As noted above, the increased hydrophobicity
analog, such as Ile^1-PPP may be synthesized as a
"homopolymer", or a "copolymer" of $-X^2-(VPGVG-)_n-Y^2-$
and $-X^1-(IPGVG-)_n-Y^1-$ may be synthesized with the molar

20   ratio of the constituent pentamers being dependent upon
the desired temperature for elastomeric force
development. However, in general, in such
"copolymers", the $-X^1-(IPGVG-)_n-Y^1-$ pentameric
component is present in about 1-99% of the total

25   pentameric molar content, while the $-X^2-(VPGVG-)_n-Y^2-$
pentameric component is present in about 99-1% of the
total pentameric molar content. More preferably, the
$-X^1-(IPGVG)_n-Y^1-$ component is present in about 5-95% of
the total pentameric molar content, while the $-X^2-$

30     $(VPGVG-)_n-Y^2-$ component is present in about 95-5% of
the total pentameric molar content. However, any
combination of relative molar amounts can be used as
dictated by the desired transition temperature and to
compensate for the effect of the addition of specific

35   cell attachment sequences and chemotactic peptides as

these sequences and peptides can shift the temperature
of the inverse temperature transition.

    Thus, in accordance with one aspect of the present
invention, is provided vascular prostheses containing
bioelastomers which contain elastomeric units
comprising tetrapeptide, or pentapeptide or units
thereof modified by hexapeptide repeating units and
mixtures thereof, wherein said repeating units comprise
amino acid residues selected from the group consisting
of hydrophobic amino acid and glycine residues, wherein
the repeating units exist in a conformation having a β-
turn which comprises a polypentapeptide unit of the
formula:

$$-X^1-(IPGVG-)_n-Y^1-$$

wherein I is a peptide-forming residue of L-isoleucine;

    P is a peptide-forming residue of L-proline;

    G is a peptide-forming residue of glycine;

    V is a peptide-forming residue of L-valine; and
wherein X is PGVG, GVG, VG, G or a covalent bond; Y is
IPGV, IPG, IP or I or a covalent bond; and n in both
formulas is an integer from 1 to 5,000; or n is 0, with
the proviso that X^1 and Y^1 together constitute a
repeating pentapeptide unit, in an amount sufficient to
adjust the development of elastomeric force of the
bioelastomer to a predetermined temperature.

    However, the present invention also relates, as
noted above, to vascular prostheses containing
bioelastomers which contain elastomeric units
comprising tetrapeptide, or pentapeptide or units
thereof modified by hexapeptide repeating units and
mixtures thereof, wherein said repeating units comprise
amino acid residues selected from the group consisting
of hydrophohic amino acid and glycine residues, wherein

the repeating units exist in a conformation having a β-
turn which comprises A) a polypentapeptide unit of the
formula:

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$$-X^1-(IPGVG-)_n-Y^1-$$

and B) a polypentapeptide unit of the formula:

$$-X^2-(VPGVG-)_n-Y^2-$$

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wherein for the above formulas,

    I is a peptide-forming residue of L-isoleucine;

    P is a peptide-forming residue of L-proline;

    G is a peptide-forming residue of glycine;

    V is a peptide-forming residue of L-valine; and

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wherein X^1 and X^2 are each PGVG, GVG, VG, G or a
covalent bond; Y^1 is IPGV, IPG, IP or I or a covalent
bond; Y^2 is VPGV, VPG, VP, V or a covalent bond; and n
in both formulas an integer from 1 to 5,000; or n in
both formulas is 0, with the proviso that X^1 and Y^1

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together, and X^2 and Y^2 together constitute a repeating
pentapeptide unit, in relative amounts sufficient to
adjust the development of elastomeric force of the
bioelastomer to a predetermined temperature, and to be
used, as necessary, to compensate for the effect of

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adding cell attachment sequences and chemotactic
peptides as noted previously.

    It should be noted that bioelastomeric polypeptide
chains containing either one or both of the above
pentapeptide repeating units can be synthesized using

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any of the pentapeptide "monomers" that are
permutations of the basic sequence. However, if the
polymer is not synthesized using the pentapeptide
"monomers", but rather is synthesized by sequential
adding of amino acids to a growing peptide, such as in

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the case of an automatic peptide synthesizer, the
designation of the repeating unit is somewhat
arbitrary. For example, the peptide H-
V(PGVGVPGVGVPGVGVPGVGV)P-OH can be considered to
consist of any of the following repeating units and end
groups: H-(VPGVG)$_4$-VP-OH, H-V-(PGVGV)$_4$-P-OH, H-VP-
(GVGVP)$_4$-OH, H-VPG-(VGVPG)$_3$-VGVP-OH, or H-VPGV-
(GVPGV)$_3$-GVP-OH, for example.

Furthermore, it is entirely possible and within
the ambit of the present invention that mixed repeating
units such as those of the formula +VPGVGIPGVG+$_n$ can be
incorporated into the bioelastomers of the present
invention.

Synthesis of the elasticity promoting and
modifying segments, which are incorporated into the
final elastomeric polypeptide, is straightforward and
easily accomplished by a peptide chemist or by standard
methods of microbial fermentation using recombinant
DNA. The resulting intermediate peptides generally
have the structure, B^1-(repeating unit)$_n$-B^2, where B^1
and B^2 represent any chemically compatible end group on
the amino and carboxyl ends of the molecule,
respectively, and n is an integer of from 2 to about
5000. Of course, when B^1 is -H and B^2 is -OH, and n is
1, the compound is either the pentapeptide H-VPGVG-OH
or H-IPGVG-OH. When n is greater than 1, the compound
intermediate is a polypentapeptide. The same will hold
true when utilizing tetrameric repeating units in the
present bioelastomers.

It should be noted that the term "hydrophobic
amino acid" refers to amino acids which have
appreciably hydrophobic R groups as measured on a
hydrophobicity scale generated by measuring the
relative solubilities of the amino acids in organic
solvents or by their relative effects on surface

tension. In this respect, see <u>Arch. Biochem. Biophy</u>,
Bull and Breese, Vol. 161, 665-670 (1974). By this
method, all amino acids which are more hydrophobic than
glycine may be used. More specifically, preferable
5   hydrophobic amino acids are Ala, Val, Leu, Ile, Pro and
Phe.
        It should also be noted that it is entirely
possible that one or more amino acid residues or
segments of amino acid residues not present in the
10   normal pentapeptide or tetrapeptide sequence may be
interspersed within a polypentapeptide or
polytetrapeptide portion of an elastomeric polypeptide
chain.

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EXAMPLES

Peptide Synthesis

The synthesis of Ile[1]-PPP was carried out by the classical solution methods as shown in Scheme I.

5   In the following Examples, the following abbreviations will be used: Boc, tert-butyloxycarbonyl; Bzl, benzyl; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; HOBt, 1-

10   hydroxybenzotriazole; IBCF, isobutyl-chloroformate; NMM, N-methylmorpholine; ONp, p-nitrophenylester; TFA, trifluoroacetic acid; PPP, $(VPGVG)_n$; Ile[1]-PPP, $(IPGVG)_n$; V, valine; I, isoleucine; P, proline; G, glycine.

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Scheme I

Synthesis of $H-(Gly-Val-Gly-Ile-Pro)_n-OH$
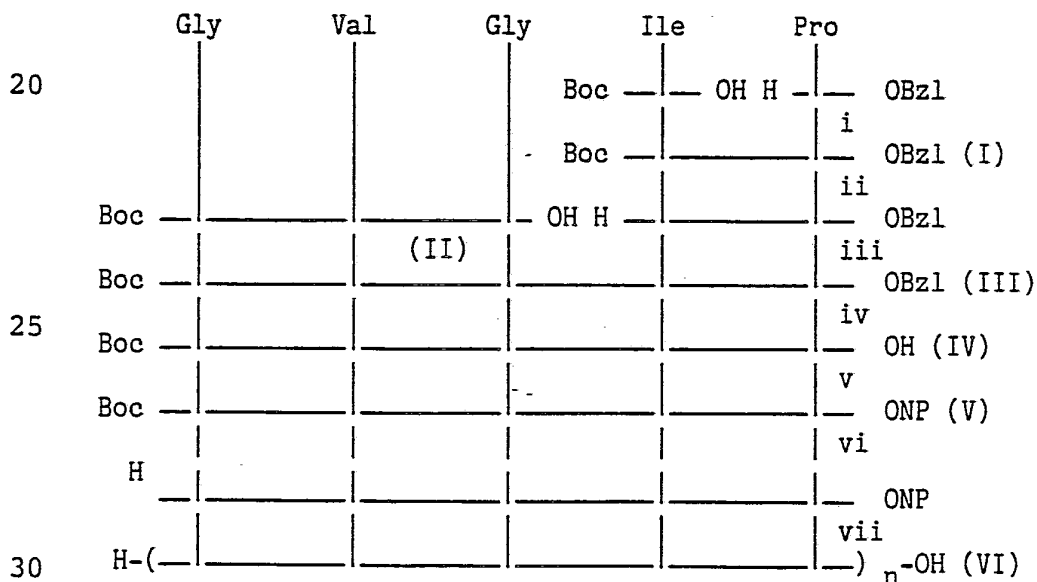
i) IBCF/HOBt; ii) HCl/Dioxane; iii) EDCI/HOBt;
iv) H2-Pd/C; v) Bis(p-nitrophenyl)carbonate;
vi) TFA; vii) DMSO-NMM

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The sequence of the starting pentamer for
polymerization is preferably Gly-Val-Gly-Ile-Pro rather
than Ile-Pro-Gly-Val-Gly, because the permutation with
Pro as the C-terminal amino acid produces high
5   molecular weight polymers in better yields. The
approach to the synthesis entailed coupling the
tripeptide Boc-GVG-OH (II) with H-IP-OBzl, each in turn
being synthesized by the mixed anhydride methodology of
J.R. Vaughan et al, J. Am. Chem. Soc., 89, 5012
10   (1967). The possible formation of the urethane as a
by-product during the reaction of Boc-Ile-OH with H-
Pro-OBzl by the mixed anhydride method was avoided by
carrying out the reaction in the presence of HOBt. The
dipeptide was also prepared using EDCI for confirmation
15   of the product. The pentapeptide benzylester (III) was
hydrogenated to the free acid (IV) which was further
converted to the p-nitrophenylester (V) on reacting
with bis(p-nitrophenyl)carbonate. On removing the Boc-
group, a one molar solution of the active ester in DMSO
20   was polymerized in the presence of 1.6 equiv. of NMM.
The polypeptide was dialyzed against water using a
50,000 dalton cut-off dialysis tubing and
lyophilized. The purity of the intermediate and final
products was checked by carbon-13 nuclear magnetic
25   resonance, elemental analyses and thin layer
chromatography (TLC).

Elemental analyses were carried out by Mic Anal,
Tucson, AZ. All amino acids are of L-configuration
except for glycine. Boc-amino acids were purchased
30   from Bachem, Inc., Torrance, CA. HOBt was obtained
from Aldrich Chemical Co., Milwaukee, WI. TLC was
performed on silica gel plates purchased from Whatman,
Inc., Clifton, NJ in the following solvent systems:
R_f^1, CHCl$_3$ (C):CH$_3$OH(M):CH$_3$COOH(A), 95:5:3, R_f^2, CMA
35   (85:15:3); R_f^3, CMA (75:25:3); R_f^4, CM (5:1). Melting

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points were determined with a Thomas Hoover melting
point apparatus and are uncorrected.

Boc-Ile-Pro-OBzl (mixed anhydride method) (I):
Boc-Ile-OH (12.01 g. 0.05 mole) in DMF (50 ml) was
cooled to 0°C and NMM (5.49 ml) was added. After
cooling the solution to -15°C isobutylchloroformate
(6.48 ml) was added slowly while maintaining the
temperature at -15°C and stirred for 10 minutes at
which time HOBt (7.65 g) was added and stirring was
continued for additional 10 minutes. A pre-cooled
solution of HCl-H-Pro-OBzl (12.09 g. 0.05 mole) in DMF
(50 ml) and NMM (5.49 ml) was added to the above
solution and the completeness of the reaction was
followed by TLC. The reaction mixture was poured into
a cold saturated $NaHCO_3$ solution and stirred for one
hour. The peptide was extracted into $CHCl_3$ and washed
with acid and base (0.5 N NaOH to remove HOBt), and on
evaporting the solvent the product was obtained as an
oil in 92% yield. R_f^1, 0.65. Anal. Calcd. for
$C_{23}H_{34}N_2O_5$: C 66.00, H 9.19, N 6.69%. Found: C 65.58,
H 8.28, N 7.13%.

Boc-Ile-Pro-OBzl (using EDCI): Boc-Ile-OH (7.20
g, 0.03 mole) and HOBt (5.05 g, 0.033 mole) in DMF (30
ml) was cooled to -15°C and EDCI (6.32 g, 0.033 mole)
was added. After stirring for 20 minutes, a pre-cooled
solution of HCl-H-Pro-OBzl (7.25 g, 0103 mole) in DMF
(30 ml) and NMM (3.3 ml) was added and stirred
overnight at room temperature. After evaporating DMF,
the residue was taken into $CHCl_3$ and extracted with 20%
citric acid and 0.5 N NaOH. The solvent was removed
and the product was obtained as an oil in almost
quantitative yield which was identical to the product
obtained by the mixed anhydride method.

Boc-Gly-Val-Gly-Ile-Pro-OBzl (III): Boc-GVG-OH
(II) (20) (5.6 g, 0.017 mole) was coupled with H-

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IlePro-OBzl (6.7 g, 0.019 mole) (obtained by deblocking
I with HCl/Dioxane) in the presence of EDCI (3.65 g,
0.019 mole) and HOBt (2.9 g, 0.019 mole) and the
product was worked up as described above to obtain
8.8 g of III (yield: 82.4%), m.p. 107-108°C
(decomp.) R_f^1, 0.44; R_f^2, 0.75. Anal. calcd. for
$C_{32}H_{49}N_5O_{10}$: C 60.83, H 7.81, N 11.08%. Found: C
61.12, H 8.06, N 11.06%

Boc-Gly-Val-Gly-Ile-Pro-OH (IV): III (7.8 g,
0.0123 mole) was taken in acetic acid (80 ml) and
hydrogenated in the presence of 10% Pd-C (1 g) at 40
psi. After filtering the catalyst with the aid of
celite, the solvent was removed under reduced pressure,
triturated with ether, filtered, washed with ether then
pet. ether and dried to obtain 6.5 g of the product
(yield: 97.3%), m.p. shrinks at 127°C and decomp. at
145°C. R_f^3, 0.24; R_f^4, 0.11. Anal. Calcd. for
$C_{25}H_{43}N_5O_{10} \cdot 1/2H_2O$: C 54.52, H 8.05, N 12.7%. Found: C
54.32, H 8.02, N 12.59%.

Boc-Gly-Val-Gly-Ile-Pro-ONp (V): IV (5.41 g, 0.01
mole) in pyridine (40 ml) was reacted with
bis(pnitrophenyl)carbonate (4.56 g, 0.015 mole)
following the completeness of the reaction by TlC.
Pyridine was removed; the residue was taken into $CHCl_3$
and extracted with acid and base. The p-nitrophenyl
ester obtained was chromatographed over a silica gel
(200-400 mesh) column. After initial washing with
$CHCl_3$, 4.8 g of V was obtained when eluted with 35%
acetone in $CHCl_3$ (yield: 71.4%), m.p. 97-100°C.
R_f^4, 0.72; R_f^4, 0.75; Anal. Calcd. for
$C_{31}H_{46}N_6O_{12} \cdot 2H_2O$: C 53:28, H 7.21, N 12.02%. Found:
C 53.76, H 6.83, N 12.01%.

H-(Gly-Val-Gly-Ile-Pro)n-OH(VI): The Boc-group
was removed from V (3.8 g, 0.0057 mole) by reacting
with TFA (35 ml) for 45 min. TFA was removed under

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reduced pressure, triturated with ether, filtered,
washed with ether, pet. ether and dried. The TFA salt
(3.3 g, 0.0049 mole) in DMSO (4.9 ml) was stirred for
14 days in the presence of NMM (0.86 ml, 0.0078
5   mole). After diluting with water in the cold, the
polypeptide was dialyzed using a 50 kD cut-off dialysis
tubing changing the water daily for 15 days. The
retentate was lyophilized to obtain 1.81 g of the Ile[1]-
polypentapeptide (yield: 88%). The carbon-13 NMR
10   spectrum was obtained to confirm the structure.
         Temperature Profiles for Coacervation
         The temperature dependence for aggregation of the
polypentapeptide is followed as the development of
turbidity at 300 nm using a Cary 14 spectrophoto-
15   meter. The sample cell is placed within a chamber
vibrating at 300 Hz in order to facilitate equilibrium
and to keep the aggregates from settling. The scan
rate is 30°C/hour and the temperature was controlled
with a Neslab ETP-3 programmer and monitored with an
20   Omega 199A thermocouple monitor placed at the cell.
The turbidity as a function of temperature provides a
temperature profile for coacervation which is found to
be concentration dependent. As the concentration is
raised, the profile shifts to lower temperatures until
25   further increases in concentration cause no further
lowering of the temperature for aggregation. This
defines the high concentration limit. The temperature
for the onset of coacervation at the high concentration
limit coincides with the temperature for the onset of
30   the transition within the coacervate itself, even when
there is no appreciable change in water content of the
coacervate. The temperature for the midpoint of the
temperature profile for the high concentration limit
has been shown to correlate with the molecular weight
35   of the polypentapeptide. When the midpoint is 25°C for

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the PPP, the molecular weight is close to 100,000
daltons as calibrated by dialysis. For the Ile^1-PPP
having a midpoint of 9°C, the molecular weight is
greater than 50,000 daltons, as the synthetic
5·   polypeptide was retained by a 50,000 daltons dialysis
membrane. The dialysis was carried out at 4°C where
the Ile^1-PPP is in solution.

Circular Dichroism Measurements

The circular dichroism studies were carried out on
10   a Cary 60 spectropolarimeter equipped with a Model 6001
CD accessory modified for 330 Hz modulation of the left
and right circularly polarized light. A concentration
of 0.025 mg Ile^1-PPP/ml of doubly distilled water was
characterized in a 10 mm path length cell. The low
15   concentration was used to keep the size of the
aggregate sufficiently small as not to cause light
scattering distortions of the CD spectra. Even at this
low concentration with this more hydrophobic
polypentapeptide, above 35°C the size of the aggregates
20   was sufficient to cause particulate distortions as was
apparent with the red shifting and dampening of the
long wavelength negative band. The temperature was
controlled and monitored from the cell as for the
temperature profiles for coacervation.

25   Formation of the Elastomeric Matrix

In preparation for γ-irradiation cross-linking
(the means of forming the elastomeric matrix), 130
milligrams of peptide Ile^1-PPP were dissolved in 220
milligrams of water in a cryotube. The sample was then
30   shear oriented at 0°C in a previously described pestle-
cryotube arrangement. Gamma-irradiation was carried
out at the Auburn University Nuclear Science Center at
a dose rate of approximately 8,000 Roentgen/min and for
sufficient time to achieve a 20×10^6 radiation
35   absorbed dose (20 Mrad).

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Thermoelasticity Studies

Thermoelasticity studies were carried out on a
stress-stain instrument built in this Laboratory. The
sample is mounted in two Delrin clamps. The top clamp
5   is attached to a Statham UTC strain-gauge and the
assembly is fixed. The bottom clamp is attached to a
moving platform driven by a variable speed motor. Both
clamps are enclosed in a thermostated water jacket. An
inner chamber contains the solvent in which the
10   elastomer is immersed which in this case is doubly
distilled water. The sample was fixed in the top clamp
and equilibrated in water at 60°C for about an hour.
The strain-gauge signal conditioner was balanced for
zero force and the bottom clamp was attached to the
15   sample. The sample was left to set overnight at room
temperature. The bottom clamp was then adjusted for
zero force and the distance between the clamps was
measured. The elastomer was elongated to 40% extension
at 5°C and elastomeric force was then determined as a
20   function of temperature. Equilibrium time to achieve
constant force at a given temperature was typically
twenty-four hours. Force measurements were made in 2°C
increments through the sharp rise in force and 5°C
increments at higher temperatures.
25

RESULTS

Temperature Profiles for Coacervation The Ile^1-PPP
can be dissolved in water on standing below 8°C. On
raising the temperature of the solution above 8°C, the
30   solution becomes cloudy; on standing at the elevated
temperature settling occurs and a viscoelastic phase
forms in the bottom of the vial; on placing the vial in
an ice bath the cloudiness immediately clears and the
viscoelastic phase readily dissolves. Thus the Ile^1-
35   PPP coacervates when dissolved in water. The

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temperature profiles for coacervation (turbidity
profiles) may be observed at different
concentrations. As the concentration is raised, the
temperature profile shifts to lower temperature. At 40

5   mg/ml, the high concentration limit (i.e, the lower
concentration for which further increases in
concentration cause no further lowering of the
temperature for the onset of aggregation), the midpoint
for the temperature profile for coacervation of Ile^1-

10   PPP is 9°C.

Notably, the simple addition of a CH_2 to the 409
dalton repeating unit causes the onset of aggregation
to shift to lower temperatures by 16°C. Observing that
the curves for (0.1 mg Ile^1-PPP/ml) and (1.0 mg PPP/ml)

15   are comparable with respect to the high concentration
limits for each high molecular weight polymer suggests
that the size of the aggregate for Ile^1-PPP is greater
for a given concentration than it is for a comparable
concentration of PPP. This will be relevant to

20   comparisons made in the circular dichroism data.

        Circular Dichroism.

        The circular dichroism curves were measured for
Ile^1-PPP in water (0.025 mg/ml) at 2°C and at 35°C.
The low concentration was chosen in order that the size

25   of the aggregate formed on association at 35°C would
have limited particulate distortions in the CD
spectrum. At low temperature there is a large negative
band near 195 nm. Such a negative band is
characteristic of disordered proteins and polypeptides,

30   though a standard value for this negative peak for
complete disorder is -4×10^4 rather than the observed
value of -1.2×10^4. Also the negative band near 220
nm, rather than zero ellipticity or a positive band
which are taken is indicative of complete disorder,

35   suggests elements of order at low temperature. The

decrease in intensity of the negative CD band near 195
nm on raising the temperature of Ile^1-PPP in water
indicates an increase in intramolecular order on
raising the temperature, that is, there is an inverse
5   temperature transition in an aqueous system. This
indicates that hydrophobic interactions are developing
as the ordered state develops. The intramolecular
increase in order begins just above 0°C and is complete
by about 30°C for a concentration of 0.025 mg/ml. The
10   transition would have been complete at a lower
temperature (the transition would have been sharper) if
the CD data could have been obtained at higher
concentration without significant particulate
distortion. Experimentally it can be demonstrated that
15   Ile^1-PPP and PPP have essentially identical
conformations below the onset temperature for the
transition and that they have essentially identical
conformations after the transition is mostly
completed. Thus while maintaining essentially
20   identical conformations, which is assisted by the
retention of β-branching, the addition of a CH_2 moiety
lowers the transition toward increased order by about
15°C.

        Characterization of Elasticity
25   The elastic (Young's) modulus determined for 20
MRAD cross-linked Ile^1-PPP coacervate was 4×10^5
$dynes/cm^2$ which is within the range of values obtained
for 20 Mrad cross-linked PPP. The range of values is
due to variable vacuolization occurring
30   during γ-irradiation which makes difficult accurate
measurement of cross-sectional area. It should be
appreciated, however, that γ-irradiation causes no
detectable polymer breakdown when measured by carbon-13
and nitrogen-15 NMR.
35   The temperature dependence of elastomeric force

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for an elastomeric band of Ile[1]-PPP at 40% elongation
is now considered. A near zero elastomeric force is
measured at 8°C; on raising the temperature there is a
dramatic and abrupt increase in elastomeric force.
Full force is reached by 25°C and becomes essentially
constant with further increases in temperature. A
similar dramatic rise in elastomeric force with
increase in temperature is observed for 20 Mrad cross-
linked PPP coacervate at 60% extension but this curve
is displaced about 15°C to higher temperatures. Using
three different physical methods it can be shown that
the addition of a CH_2 moiety (the replacement of Val by
Ile) shifts the transition to lower temperatures by
15°C without changing the conformation of the
polypentapeptide before and after the transition.
While the previously reported data on the naturally
occuring PPP of elastin demonstrate a correlation of
increased structural order with increased elastomeric
force, the Ile[1]-PPP data with the transition shifted by
15°C appear to confirm an obligatory coupling of
increased order with increase elastomeric force at
fixed length.

In fact, the correlation of increased order with
increased elastomeric force at fixed length is seen
with the PPP. When the transition is shifted to lower
temperatures, as in Ile[1]-PPP, the development of
elastomeric force faithfully shifts to lower
temperatures. There appears in such elastomeric
polypeptides to be a strict coupling between increasing
order and increasing elastomeric force; and the
molecular structure provides an understanding as to how
this can occur. The similar conformations of PPP and
Ile[1]-PPP and the similar elastic moduli for the two
polymers indicate that these do not appear to be
factors in the evolutionary retention of $(VPGVG)_n$.

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What is now clear is that even the subtle addition of
a-CH$_2$-moiety, for example, while having little effect
on the stereochemistry of rather nonexacting,
nonrestricting hydrophobic associations, has a
significant effect on the thermodynamics. The larger
clathrate-like cage of water surrounding the Ile side
chain provides a greater ΔS as the more-ordered water
surrounding the side chain becomes less ordered bulk
water such that in the transition $\Delta H = T\Delta S$ at a lower
temperature. By means of calorimetry, the ΔH for PPP
has been estimated at 2 to 3 cal/gram which is
approximately 1 kcal/mole of pentamers. Thus, the
increase in entropy change need only be about 5% to
cause the temperature of the transition to decrease
about 15°C from 298°K to 283°K. Utilizing known
hydrophobicity scales for amino acids, the
hydrophobicities given in a free energy of transfer
scale of kcal/mole, are -4.10 for VPGVG and -5.38 for
IPGVG. While the extent of the hydrophobicity that is
utilized is expected to depend on the stereochemistry
of the more-ordered polypeptide state, it would appear
that not all of the total potential effect is actually
realized.

The above-described hydrophobic effect upon
transition temperatures is also supported by the
elastin polytetrapeptide, (Val^1-Pro^2-Gly^3-Gly^4)$_n$. That
is, it has also been discovered that high molecular
weight PTP undergoes a reversible temperature elicited
aggregation with an onset of aggregation at 48°C,
rather than 24°C as for high molecular weight PPP.

However, it has also been found that the inverse
temperature transition for PTP is only complete at
about 70°C. Moreover, this high temperature of
transition appears to be explained by considering the
lower hydrophobicity of PTP as compared to PPP.

For example, utilizing the Nazaki-Tanford and
Bull-Breese hydrophobicity scales with the
hydrophobicity of the Gly residue taken as zero, the
free energy of transfer for the pentamer, VPGVG, would
be -4100 cal/mole whereas that of the tetramer, VPGG,
would be -2540 cal/mole. Thus, if hydrophobicity of
the repeating unit is the determining factor, then the
inverse temperature transition for the PTP would be at
a higher temperature than that of the PPP. Furthermore
if the inverse temperature transition (the increase in
intramolecular order) is required for the development
of elastomeric force, then the temperature dependence
of elastomeric force of the PTP matrix would be
expected to show a similar shift to higher temperature
relative to that of the PPP matrix.

This inverse temperature transition is actually
centered at near 50°C for PTP, shifted some 25°C higher
than that of PPP. For Ile[1]-PTP, it is shifted some
30°C lower in temperature than that of PTP. Also, it
has been found that the development of elastomeric
force upon raising the temperature is similarly shifted
about 25°C higher for the PTP matrix (20 Mrad cross-
linked) as compared to the PPP matrix (20 Mrad
crosslinked).

Accordingly, in view of the above, it is now
possible, by selecting the appropriate combination of
PTP and PPP matrices or analogs thereof of the present
invention to shift the transition temperature of a
bioelastomer containing elastin PTP, PPP and analogs
thereof and PHP over a range of about 75°C.
Furthermore, wherever this transition would occur in
the range of about -25°C for Phe[1]-PPP in water/ethylene
glycol or about 50°C for PTP, in water, for example,
there is a large change in elastomeric force which
accompanies a relatively small change in temperature.

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Thus, it is now possible to provide bioelastomers having incorporated therein repeating units having decreased hydrophobicity, such as-(VPGG)$_n$-.

In particular, in accordance with the present invention, is also provided a bioelastomer containing elastomeric units comprising tetrapeptide, or pentapeptide or units thereof modified by hexapeptide repeating units and mixtures thereof, wherein the repeating units comprise amino acid residues selected from the group consisting of hydrophobic amino acid and glycine residues, wherein said repeating units exist in a conformation having a β-turn which comprises a tetrapeptide of the formula:

$$-X^3-(VPGG)_n-Y_3-$$

wherein X^3 is PGG, GG, G or a covalent bond;

Y^3 is VPG, VP, V or a covalent bond; and

V is a peptide-producing residue of L-valine;

P is a peptide-producing residue of L-proline; and

G is a peptide-producing residue of glycine;
and n is an integer from 1 to 5,000, or n is 0, with the proviso that X^3 and Y^3 together constitute a repeating tetrameric unit in an amount sufficient to adjust the development of elastomeric force of the bioelastomer to a predetermined temperature.

Moreover, the materials of the present invention also further provides a bioelastomer containing elastomeric units comprising tetrapeptide, or pentapeptide or units thereof modified-by hexapeptide repeating units and mixtures thereof, wherein the repeating unit comprises amino acid residues selected from the group consisting of hydropholic amino acid and

glycine residues, wherein said repeating units exist in
a conformation having a β-turn which comprises

    A) a polypentapeptide of the formula:

$$-X^1-(IPGVG)_n-Y^1-$$

wherein X^1, Y^1, P, G, I, V and n are as defined above;
and

    B) a polypentapeptide of the formula:

$$-X^2-(VPGVG)_n-Y^2-$$

wherein X^2, Y^2, P, G, V and n are as defined above; or

   C) a polytetrapeptide of the formula:

$$-X^3-(VPGG)_n-Y^3-$$

wherein X^3, Y^3, P, G, V and n are as defined above in
relative amounts sufficient to adjust the development
of elastomeric force of said bioelastomer to a
predetermined temperature, and to compensate for the
effect of adding cell attachment sequences and
chemotactic peptides.

    In accordance with the present invention are also
provided PTP analogs, such as Ile^1-PTP, which are
analogous to the various PPP analogs described above.
In fact, any PTP-analog can be used in the preparation
of the present bioelastomers which suffices to
attenuate the hydrophobicity of the functional
repeating unit, such as $(IPGG)_n$, while retaining the
elasticity of the bioelastomer and other properties
noted above. Accordingly, in view of the principles
set out above, one skilled in the art would, in view of
this disclosure, be able to ascertain other PTP analogs
which can be used advantageously in accordance with the

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present invention.

Thus, in accordance with the present invention is also provided vascular prosthesis containing a bioelastomer containing elastomeric units comprising tetrapeptide, or pentapeptide or units thereof modified by hexapeptide repeating units and mixtures thereof, wherein the repeating units comprise hydrophobic amino acid and glycine residues, wherein the repeating units exist in a conformation having a β-turn which comprises a tetrapeptide of the formula:

$$-X^4-(IPGG)_{\overline{n}}Y^4-$$
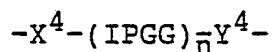
wherein X^4 is PGG, GG, G or a covalent bond;

Y^4 is IPG, IP, I or a covalent bond; and

I is a peptide-producing residue of L-isoleucine;

P is a peptide-producing residue of L-proline; and

G is a peptide-producing residue of glycine; and n is an integer from 1 to 5,000, or n is 0, with the proviso that X^4 and Y^4 together constitute a repeating tetrameric unit, in an amount sufficient to adjust the temperature of which the elastomeric force of the bioelastomer develops, and to compensate for the effect of adding cell attachment sequences and chemotactic peptides.

Of course, the materials used in the present invention are bioelastomers having the above-recited structural features, but which have any combination of the repeating units $(IPGVG)_{\overline{n}}$, $(VPGVG)_{\overline{n}}$, $(VPGG)_{\overline{n}}$, $(IPGG)_n$ or other analogs thereof, such as Ala^3-PPP or Phe^1-PPP.

In fact, the present invention includes, in general, all vascular prosthesis containing bioelastomers containing elastomeric units comprising tetrapeptide, or pentapeptide or units thereof modified

by hexapeptide repeating units and mixtures thereof,
wherein the repeating units comprise hydrophobic amino
acid residues and glycine residues, wherein the
repeating units exist in a conformation having a β-turn
5   which comprises a tetrapeptide or pentapeptide unit or
repeating unit thereof, in an amount sufficient to
adjust the development of elastomeric force of said
bioelastomer to a predetermined temperature, with the
proviso that the elasticity of the bioelastomer is
10 retained.

        However, in order to clarify the various aspects
of the present invention relating to PTP, the following
Examples and discussion are provided. Of course, the
Examples are for purposes of illustration only and are
15 not intended to limit the present invention.

EXAMPLES
        Peptide Synthesis
        General Approach: The synthesis of polytetra-
20 peptide, $(VPGG)_n$, can be achieved using any of the
following permutations as the starting tetramer unit:
Val-Pro-Gly-Gly, Gly-Val-Pro-Gly, Gly-Gly-Val-Pro, or
Pro-Gly-Gly-Val. The first sequence (VPGG) was used in
this laboratory both with the pentachlorophenyl ester
25 (OPcp) activation and with the p-nitrophenyl ester
(ONp) activation methods, and the latter method yielded
polymer of significantly higher molecular weight. In
synthesizing the polypentapeptide, $(VPGVG)_n$, using
different permutations of the pentamer unit with
30 different activating groups for polymerization, it was
observed that the pentamer having Pro as the C-terminal
amino acid and -ONp for activation gave high molecular
weight polymers. Similar results have been experienced
in the case of the preparation of polyhexapeptide,
35 $(VAPGVG)_n$. Hence, a similar approach was determined to

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be reasonable in the case of PTP also, i.e., sequence
(GGVP) with -ONp activation. For comparison,
H-VPGG-ONp, H-GVPG-ONp and H-GGVP-ONp were all tried
for polymerization. As expected, the latter tetramer
sequence gave a very high molecular weight polymer when
determined by the TP τ-studies and here is described
the synthesis of this latter material as shown in the
Scheme II. The sequence (PGGV) was not attempted
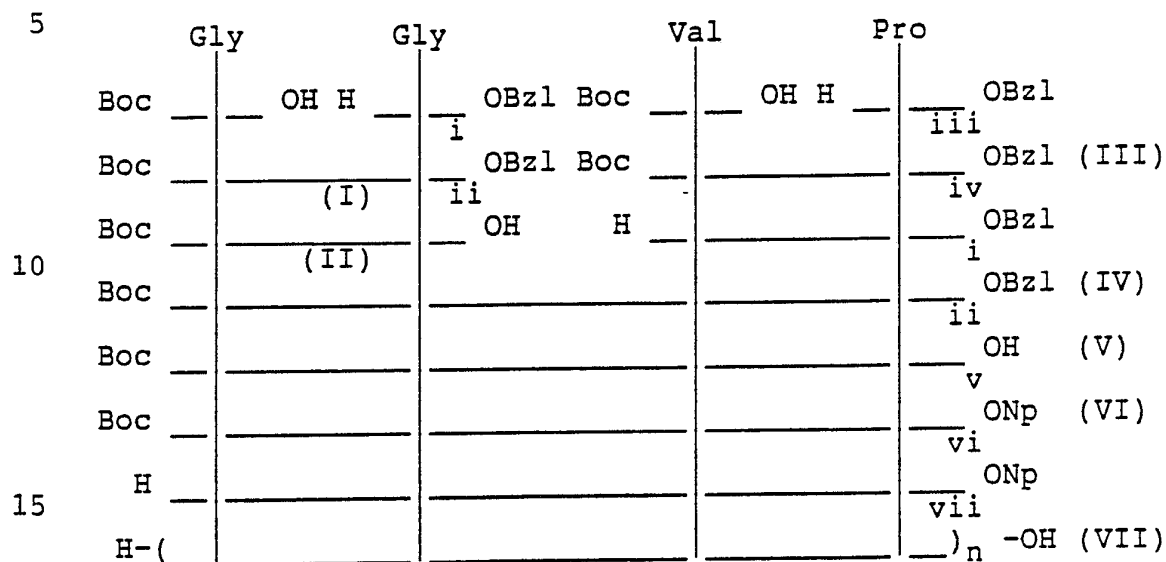because it has an optically active and bulky amino
acid, Val, at its C-terminal.

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Scheme II

Synthesis of H-(Gly-Gly-Val-Pro)$_n$-OH

	Gly	Gly	Val	Pro	
Boc	OH H	OBzl Boc	OH H	OBzl	
		i		iii	
Boc		OBzl Boc		OBzl	(III)
	(I)	ii		iv	
Boc		OH H		OBzl	
	(II)			i	
Boc				OBzl	(IV)
				ii	
Boc				OH	(V)
				v	
Boc				ONp	(VI)
				vi	
H				ONp	
				vii	
H-(				)$_n$ -OH	(VII)

i) EDCI-HOBt; ii) H$_2$-Pd/C; iii) IBCF-HOBt;
iv) HCl/Dioxane v) Bis(p-nitrophenyl)carbonate;
vi) TFA; vii) DMSO-NMM

Boc-GG-OBzl (I) was prepared using EDCI for coupling and was hydrogenated to give the acid (II). Boc-VP-OBzl (III) was synthesized by the mixed anhydride method in the presence of HOBt, deblocked, and coupled with II using EDCI-HOBt to obtain Boc-GGVP-OBzl (IV). After hydrogenating to the acid, V, it was converted to -ONp (VI) by reacting with bis(p-nitrophenyl)carbonate. After removing the Boc-group, the active ester was polymerized, dialyzed against water using a 50,000 molecular weight cut-off dialysis tubing and lyophilized. The intermediate and the final products were checked by carbon-13 nuclear magnetic resonance, thin-layer chromatography (TLC) and elemental analyses.

Details of Syntheses: Valine and Proline are of

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L•configuration. Boc-amino acids were purchased from
Bachem, Inc., Torrance, CA. HOBt was obtained from
Aldrich Chemical Co., Milwaukee, WI, and Bio-sil silica
gel (200-400 mesh) was purchased from Bio-Rad
Laboratories, Richmond, CA. TLC plates were obtained
from Whatman, Inc., Clifton, NJ and the following
solvent systems were used for determining the
homogeneity of the products: R_f^1, CHCl$_3$(C):MeOH
(M):CH$_3$COOH (A), 95:5:3; R_f^2, CMA (85:15:3); R_f^3, CMA
(75:25:3); R_f^4, CM (5:1). Elemental analyses were
carried out by Mic Anal, Tuscon, AZ. Melting points
were determined with a Thomas Hoover melting point
apparatus and are uncorrected.

Boc-Gly-Gly-OBzl (I): Boc-Gly-OH (17.52 g, 0.1
mole) in a mixture of CHCl$_3$ (50 ml) and acetonitrile
(50 ml) was cooled to -15°C and EDCI (19.17 g, 0.1
mole) was added and stirred for 20 minutes. To this, a
pre-cooled solution of H-Gly-OBzl•tosylate (37.1 g,
0.11 mole), NMM (12.09 ml, 0.11 mole) in CHCl$_3$(100 ml)
was added and stirred overnight at room temperature.
After removing the solvent, the residue was taken in
CHCl$_3$ and extracted with acid and base. Chloroform was
removed under reduced pressure, triturated with pet.
ether, filtered, washed with pet. ether and dried to
obtain 30.2 g of I (yield: 93.7%), m.p. 82-83°C. R_f^2,
0.52; R_f^4, 0.82. Anal. Calcd. for C$_{16}$H$_{22}$N$_2$O$_5$: C,
59.61; H, 6.88; N, 8.69%. Found C, 59.43; H, 6.88; N,
8.35%

Boc-Gly-Gly-OH (II): I (10 g, 0.31 mole) in
acetic acid (100 ml) was hydrogenated at 40 psi in the
presence of 10% Pd-C catalyst (1 g). The catalyst was
filtered with the aid of celite and solvent removed
under reduced pressure. The residue was triturated
with EtOAC, filtered, washed with EtOAC, pet. ether and
dried to yield 6.3 g of II (yield: 87.5%), m.p.

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118-120°C (decomp.). R_f^2, 0.28; R_f^3, 0.44. Anal. Calcd.
for $C_9H_{16}N_2O_5 \cdot H_2O$: C, 43.19; H, 7.25; N, 11.19%.
Found: C, 43.53; H, 7.40; N 10.90%.

Boc-Gly-Gly-Val-Pro-OBzl (IV): III (6.0 g, 0.0148
mole) (39) was deblocked with HCl/Dioxane and solvent
removed under reduced pressure. The residue was
triturated with ether, filtered, washed with ether,
then pet. ether and dried. A very hygroscopic material
was obtained (4.2 g, 0.0123 mole) which was coupled in
DMF with II (2.86 g, 0.0123 mole) in the presence of
10% excess of EDCI (2.60 g) and HOBt (2.07 g). The
reaction was worked up as described for I to obtain IV
as a white foam in a quantitative yield, no sharp m.p.
54-62°C. R_f^2, 0.42; R_f^3, 0.74. Anal. Calcd.
for $C_{26}H_{38}N_4O_7$; C, 60.21; H, 7.38; N, 10.80%. Found:
C, 60.0; H, 7.46; N, 10.81%.

Boc-Gly-Gly-Val-Pro-OH (V): IV (6.2 g, 0.012
mole) in acetic acid was hydrogenated and worked up as
for II to obtain V quantitatively, no sharp m.p.
74-83°C. R_f^3, 0.25; R_f^4, 0.15. Anal. Calcd. for
$C_{19}H_{32}N_4O_7$: C, 51.10; H, 7.67; N, 12.54%. Found: C,
51.28: H, 7.50 N, 12.38%.

Boc-Gly-Gly-Val-Pro-ONp (VI): V (5.3 g, 0.0123
mole) in pyridine (30 ml) was reacted with
bis(p-nitrophenyl)carbonate (5.64 g, 0.0185 mole).
After removing the solvent, the residue was taken in
$CHCl_3$ and extracted with acid and base. The peptide
was chromatographed over a silica-gel column and eluted
with 35% acetane in $CHCl_3$ after initially eluting with
$CHCl_3$, to obtain 4.7 g of VI (yield: 69.2%), no sharp
m.p. 74-79°C. R_f^2, 0.76; R_f^4, 0.75. Anal. Calcd. for
$C_{25}H_{35}N_5O_9 \cdot 1/2H_2O$: C, 53.75; H, 6.49; N, 12.53%.
Found: C, 53.69; H, 6.44; N, 12.34%.

H-(Gly-Gly-Val-Pro)$_n$-OH (VII): VI (4.5 g, 0.0082
mole) in $CHCl_3$ (20 ml) was treated with TFA (35 ml) for

30 minutes and solvent removed under reduced
pressure. The residue was triturated with ether,
filtered, washed with ether, then with pet. ether and
dried. The TFA salt (3.9 g, 0.0069 mole) in DMSO (7.6

5   ml) and NMM (1.22 ml, 1.6 equiv) was stirred for 14
days. After diluting with cold water, the polymer was
dialyzed in a 50 kD cut-off dialysis tubing, changing
water daily for 15 days, and the retentate was
lyophilyzed to yield 1.65 g of the polytetrapeptide

10   (yield: 77%). A carbon-13 NMR spectrum of the polymer
was obtained and there were no extraneous peaks thereby
verifying the synthesis and purity of the product.

       Temperature Profiles for Coacervation

15   Polypeptide coacervation in water is reversible
aggregation to form a new phase with a distinct
composition. Association occurs on raising the
temperature, disassociation on lowering the
temperature. The process of coacervation was followed

20   by monitoring the turbidity as a function of
temperature using a Cary 14 spectrophotometer set at
300 nm, a Neslab ETP-3 temperature programmer with a
30°C/hour scan rate and an Omega 199A thermocouple
monitor. The sample cell was placed in a vibrating

25   chamber (300 Hz) to keep the aggregates from settling
and to facilitate equilibrium. The temperature
profiles for coacervation are concentration
dependent. Dilution from a high concentration, after
the high concentration limit is reached (approximately

30   40 mg/ml for high molecular weight elastomeric
polypeptides), results in a shift of the turbidity
profile to higher temperature.

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Circular Dichroism Measurements

A Cary 60 spectropolarimeter equipped with a Model
6001 circular dichroism accessory with 330 Hz
modulation of the left and right circular polarized
beams was used to determine the circular dichroism
patterns of 5 mg PTP in one ml of deionized-distilled
(quartz immersion heater) water. Because of the
smaller size or the relative transparency of the PTP
aggregates (as with the cross-linked PTP matrix with a
relatively small change in refractive index between
solution and matrix) when compared to that of the PPP
system, it was possible to use the 5 mg/ml
concentration for the CD studies without being
compromised by light scattering (particulate)
distortions of the CD spectra. This is apparent from
monitoring the negative band near 220 nm which becomes
damped and red-shifted as the particulate distortions
become significant.

Preparation of the Cross-linked PTP Matrix

The PTP was prepared for γ-irradiation cross-
linking by dissolving 130 milligrams of the peptide in
220 milligrams of water in a cryotube. The material
was shear oriented overnight at 40°C in a previously
described pestle-cryotube assembly. The sample was
exposed to approximately 8,000 Roentgen/min γ-
irradiation at the Auburn University Nuclear Science
Center. Exposure was of sufficient time to achieve a
20×10^6 radiation absorbed dose (20 Mrad).

Thermoelasticity Measurements

Thermoelasticity studies were carried out on a
stress-strain apparatus. Clamping of the sample in the
holder was done in two stages to prevent damage to the
material at the clamp edge. The sample was first

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gripped lightly with the top clamp, raised to 60°C
while submerged in water within the temperature jacket
and allowed to equilibrate for about 2 hours. The
measured force consisting of the weight of the sample
5   and grips in water were set to zero. The bottom grip
was then attached to the sample and both grips
tightened to hold the sample firmly. The bottom clamp
was driven as in a stress-strain measurement and
stopped at 40% elongation. Force data were recorded in
10   5°C steps starting at 70°C and continuing to 40°C where
the force approached zero.

RESULTS:

        Temperature Profiles for Coacervation
15   The polytetrapeptide is soluble in water in all
proportions below 40°C. On raising the temperature
above 40°C the solution becomes turbid; on standing
settling occurs to form a dense viscoelastic phase
called a coacervate. The process is readily
20   reversible; on lowering the temperature cloudiness
clears and coacervate readily redissolves. By
following the turbidity as a function of temperature,
temperature profiles for coavervation are obtained
which are concentration dependent. As more
25   concentrated solutions are used, the onset of turbidity
occurs at lower temperatures until further increases of
concentration cause no further lowering of the
temperature for onset of turbidity. The lower
concentration above which raising the concentration no
30   further lowers the temperature for onset of turbidity
is called the high concentration limit. For this high
molecular weight PTP the high concentration limit is 40
mg/ml as 100 mg/ml gives the same profile. Dilution
from 40 mg/ml causes a shift to higher temperature for
35   the onset. The midpoint for the high concentration

limit of PTP is 49°C whereas the value for the high
concentration limit of PPP is 25°C. The decreased
hydrophobicity of the tetramer results in a 24°C
increase in the temperature required to bring about the
5 hydrophobic interactions attending aggregation.

    Circular Dichroism
        The CD spectra were measured at 40°C and 65°C for
5 mg/ml of PTP in water. At the lower temperature
10 there is a negative band near 220 nm and a second
negative band in the 195-200 nm range. This latter
band is considered to be indicative of polypeptides
with limited order as fully disordered polypeptides are
considered to have a negative band near 195 nm with an
15 ellipticity of -4×10^4. The lower magnitude of the
short wavelength negative band for PTP and the negative
band near 220 nm indicate some order in the PTP at
35°C. On raising the temperature the short wavelength
negative band decreases in magnitude indicative of a
20 transition toward greater intramolecular order.
Interestingly, its midpoint corresponds approximately
to the midpoint in the temperature profile for
coacervation for a comparable concentration. It is
important to note for the PTP that the change in
25 intramolecular order precedes the intermolecular
interactions, i.e., begins at a substantially lower
temperature than the aggregational process. Thus, the
intramolecular ordering of the PTP is shifted to higher
temperature due to the decreased hydrophobicity of the
30 tetramer as compared to the pentamer.

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Thermoelasticity Data

The temperature dependence of elastomeric force (thermoelasticity data) was measured for 20 Mrad cross-linked PTP at an extension of 40%. Very little elastomeric force was exhibited by this matrix below 40°C. As the temperature is raised above 40°C, however, the elastomeric force develops to a maximal value near 70°C. A 20 Mrad cross-linked PPP matrix exhibited a similar transition but at about 20° to 25°C lower in temperature. The development of elastomeric force, just as the temperature dependence of coacervation and of ellipticity for the PTP, is shifted by about 25°C from that of the PPP. These properties are a function of the hydrophobicity of the repeating unit. Of particular interest is the comparison of the ellipticity data for the PTP with the thermoelasticity for the PTP. The transition as followed by ellipticity, which is a measure of intramolecular order, begins in the range 35° to 40°C, and similarly the elastomeric force begins to develop just below 40°C. By both physical measurements the transition is essentially complete by 70°C. At fixed length, there is a close parallel between increase in intramolecular order and increase in elastomeric force. As the aggregational intermolecular processes, followed by turbidity, do not become significant until nearly 50°C, it appears that the PTP matrix allows a delineation between intramolecular and intermolecular processes as related to origins of elastomeric force.

The structural features of PTP appear to be very similar to those of PPP. For example, it is clear that the same principles are operative as for the PPP. The Type II Pro^2-Gly^3 β-turn is the dominant secondary structural feature and the ordering process is that of an inverse temperature transition with the optimization

of intramolecular hydrophobic interactions as the
temperature is raised. The perspective is again an
open helix with β-turn spacers between turns of the
spiral and with the Val and Pro side chains providing

5   the intramolecular hydrophobic contacts. The suspended
segment will necessarily be shorter and the librational
motion will be focused on the Gly^4-Val^1 peptide
moiety. Based on the cyclic conformational correlate
there will be approximately 4 tetramers per turn of

10 PTP β-spiral as opposed to the approximately 3
pentamers per turn for the PPP β-spiral.

Effect of Repeat Unit Hydrophobicity
That the transitions toward increased elastomeric

15 force are actually inverse temperature transitions
dependent on the hydrophobicity of the constituent
peptide is apparent from the direction of the shift of
the transition on changing the hydrophobicity of the
repeating unit. As the repeating unit becomes more

20 hydrophobic, the temperature for the transition shifts
to lower values. Using the Nozaki-Tanford-Bull-Breese
hydrophobicity scale, the pentamer (VPGVG) would have a
free energy for transfer of -4100 cal/mole whereas that
for the tetramer (VPGG) would be -2540 cal/mole. For

25 the transition $\Delta H = T\Delta S$, and for a given ΔH a higher
temperature would be required if the hydrophobicity
giving rise to ΔS were less. The data show that the
decreased hydrophobicity of the tetramer requires a
higher temperature for the transition than for the more

30 hydrophobic pentamer. This finding is in accordance
with the above-mentioned results obtained with Ile^1-
PPP. When $(IPGVG)_n$, or Ile^1-PPP, is prepared, the
Ile^1-PPP coacervates; it increases intramolecular order
on increasing the temperature and the Ile^1-PPP matrix

35 increases elastomeric force on raising the temperature

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but the transition is shifted to 9°C. The
hydrophobicity for this pentamer, (IPGVG), is -5380
cal/mole. A comparison of the temperature of the
inverse temperature transition for the three
5   polypeptide elastomers and the hydrophobicities of the
repeating unit illustrates a proportional relationship
between repeat unit hydrophobicity and the temperature
of the inverse temperature transition. Not only is the
direction of the shift correct but the magnitude of the
10 shift is also approximately correct. It is clear that
the inverse temperature transition giving rise to the
intramolecular ordering and elastomeric force develop-
ment is indeed proportional to the hydrophobicity of
the repeating unit, and it is the intramolecular
15 process utilizing hydrophobic interactions that is
responsible for the development of elastomeric force.
        Thus, the bioelastomers of the present invention
can encompass a wide variety of functional repeating
units in order to provide a wide variation in
20 temperature of elastomeric force development, and to
compensate for the effect of adding specific cell
attachment sequences and chemotactic peptides as noted
previously.
        For example, the present bioelastomers include
25 those having any of the following repeating units as
defined above:

$$-X^1-(IPGVG)_n-Y^1$$
$$-X^2-(VPGVG)_n-Y^2-$$
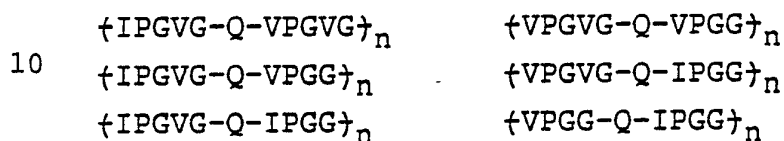$$-X^3-(VPGG)_n-Y^3-$$
30 $$-X^4-(IPGG)_n-Y^4-$$
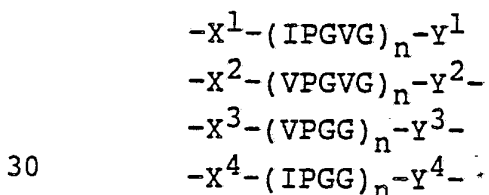
alone or in combination with each other in order to
impart to the bioelastomer a capability of developing
near maximum elastomeric force at a predetermined
temperature.
35   However, also included with in the ambit of the

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present invention are all analogs of PPP and PTP and
combinations thereof which modulate the hydrophobicity
of the PPP and PTP repeating unit or units, without
unduly interfering with either the formation of the
5   viscoelastic phase or the librational motion of the
polypeptide, i.e., the elasticity.

Other examples of such analogs and combinations
thereof are such sequences as:

$\{IPGVG\text{-}Q\text{-}VPGVG\}_n$ $\{VPGVG\text{-}Q\text{-}VPGG\}_n$

10   $\{IPGVG\text{-}Q\text{-}VPGG\}_n$. $\{VPGVG\text{-}Q\text{-}IPGG\}_n$

$\{IPGVG\text{-}Q\text{-}IPGG\}_n$ $\{VPGG\text{-}Q\text{-}IPGG\}_n$

where Q is either a direct covalent bond or an
interspersing amino acid residue or residues, which can
be any such residue which does not interfere with the
15   elasticity of the polypeptide.

Of course, the repeating pentapeptide sequence, as
well as the repeating tetrapeptide sequence can be
widely substituted to modify the repeating unit
hydrophobicity, as long as the elasticity of the
20   bioelastomer is retained. For example, the
incorporated tetrapeptide repeating unit can be of the
general formula:

$$\{R_1PR_2R_3\}_n$$
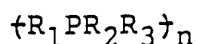
wherein R_1 is a peptide-producing residue selected from
25   the group of Phe, Leu, Ile and Val; R_2 is such a
residue selected from the group of Ala and gly; and R_3
is selected from the group consisting of Phe, Leu, Ile
and Val; and n is an integer of from 1 to about 200;
and wherein P is a L-proline-producing residue and G is
30   a glycine-producing residue. Thus, "homopolymers" of
the above tetrameric sequence can be utilized or
"copolymers" of the above sequence can be used in
conjunction with other repeating units in keeping with
this invention.

35   Also, in general, tetrapeptide repeating units of

the formula:

$$\{R_1 PGG\}_n$$
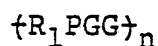
can be utilized, wherein R_1 and n are as defined above
for the pentameric sequences. These units are
incorporated into the present bioelastomers in an
amount sufficient to adjust the development of
elastomeric force of the bioelastomer to a
predetermined temperature, and to compensate for the
effect of adding specific cell attachment sequences and
chemotactic peptides.

Generally, in accordance with any of the
bioelastomers of the present invention, the
bioelastomers can be a "homopolymer" of a functional
repeating unit, such as Phe^1-PPP, Ala^3-PPP Ile^1-PPP, or
Ile^1-PTP; or they can be a "copolymer" of the general
formula $\{S_a - T_b\}_n$ wherein either S or T constitutes a
functional repeating unit designed to modify or shift
the temperature of elastomeric force development of the
bioelastomer, while either S or T, whichever is
remaining, constitutes another repeating unit of the
bioelastomer. As noted, such "copolymers" can be of
either the block or random variety, which means that a
and b can each be 1 or a larger integer.

Further, for these "copolymers", it is possible,
as noted, that more than one functional repeating unit
can be used to modify the temperature of elastomeric
force development. Thus, both units -S- and -T- in the
formula above would be such repeating units, for
example, $\{IPGVG\}$ and $\{VPGVG\}$. Of course, each of S and
T may be comprised of a subset of repeating units of
S_i, S_{ii} and S_{iii}. For example, three S subsets might
be PPP analogs, such as (IPGVG), (FPGVG), where F is
the one letter abbreviation for Phe, or (VPAVG).

Each one of the S or T repeating units is
preferably incorporated within the molar range of
1-99%. More preferably still, is the incorporation of
these units within the molar range of 5-95%. However,
5   the actual molar content of any number of different
repeating units is directly proportional to the desired
transition temperatures using hydrophobicity scales.

The bioelastomeric material of the present
invention may also contain PHP and variants thereof in
10 order to accentuate modulus of elasticity and strength
of materials produced containing these segments. This
will now be described.

Additionally, as noted above, the natural
hexapeptide and permutations of this sequence can be
15 used to control the modulus of elasticity and tensile
strength of artificial elastomers based on the
polypenta- and polytetrapeptide sequences. Thus, they
found that elastic modulus and tensile strength of the
artificial bioelastomers of the type described herein
20 can be increased by incorporating a segment of the
formula

$$-X-(APGVGV)_n-Y-$$

wherein A is a peptide-forming residue of L-alanine;
P is a peptide-forming residue of L-proline;
25   G is a peptide-forming residue of glycine;
V is a peptide-forming residue of L-valine;
X is PGVGV, GVGV, VGV, GV, V, or a covalent
bond;
Y is APGVG, APGV, APG, AG, A, or a covalent
30 bond; and

n is an integer from 2 to 5,000 wherein this
"hexameric" segment contains at least 18 amino acid
residues, into an elastomeric peptide chain. In this
way the modulus of elasticity and tensile strength can
35 be readily increased.

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The bioelastomeric polypeptide chains containing hexapeptide repeating units can be synthesized using any of the hexapeptide "monomers" that are permutations of the basic sequence. Thus, polymers generally will have the structure B^1(hexapeptide repeating unit)$_n$-B^2 where B^1 and B^2 represent the remainder of a peptide chain which is discussed later in detail. In fact, if the peptide polymer is not synthesized using hexapeptide "monomers" but rather is synthesized by sequential adding of amino acids to a growing peptide (such as in an automatic peptide synthesizer) the designation of a repeating unit is somewhat arbitrary. For example, the peptide H-V(APGVGVAPGVGVAPGVGVAPGVGV)A-OH can be considered to consist of any of the following repeating units and end groups: H-(VAPGVG)$_4$-VA-OH, H-V-(APGVGV)$_4$-A-OH, H-VA(PGVGVA)$_4$-OH, H-VAP-(GVGVAP)$_3$-GVGVA-OH, H-VAPG(VGVAPG)$_3$-VGVA-OH, or H-VAPGV-(GVAPGV)$_3$-GVA-OH.

Synthesis of the elasticity-modifying hexameric segment (which is incorporated into the final elastomeric peptide) is straight-forward and easily accomplished by a protein chemist or using standard methods of microbial fermentation with recombinant DNA. The resulting intermediate peptides generally have the structure B^3-(repeating unit)$_n$-B^4 where B^3 and B^4 represent any chemically compatible end group on the amino and carboxyl ends of the molecule, respectively, and n is an integer from 1 to about 5,000. When B^3 is H, B^4 is OH, and n = 1, the compound is the hexapeptide itself. When n is greater than 1, the compound intermediate is a polyhexapeptide. It is possible that one or more amino acid residue or segment of amino acid residues not present in the normal hexapeptide sequence may be interspersed within a polyhexapeptide portion of an elastomeric polypeptide chain. As clearly indicated

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by the previous general formula and by the following
discussion, the invention encompasses incorporation of
a hexamer or polyhexapeptide into a larger peptide
chain.

5   Other examples of terminal B^3 and B^4 end groups
for the intermediate hexameric segment that is to be
incorporated into the elastomeric polypeptide chain
include portions of the repeating peptide units
themselves with free amino or carboxylic acid groups or

10   salts thereof, free amino or carboxylic acid groups or
salts (especially alkali metal salts), and peptide or
amino acid units that have retained a blocking group
that was present during synthesis of the polypeptide or
that have a biocompatible group added after formation

15   of the polypeptide. Examples of blocking groups
include t-butyloxycarbonyl, formyl, and acetyl for the
amino end of the molecule and esters, such as methyl
esters, as well as amides, such as the amides of
ammonia and methyl amine, for the acid end of the

20   molecule.

        The first product obtained in the synthesis of a
hexapeptide monomer that is typically produced in the
process of preparing a hexapeptide segment is a
protected hexapeptide, such as Boc-L·Val-L·Ala-

25   L·Pro-Gly-L·Val-Gly-OBzl. This protected monomer is
converted into a reactive monomer that can be
incorporated into a peptide chain by, for example,
replacement of the benzyl ester with the p-nitrophenyl
ester, for example by effectively exchanging with p-

30   nitrophenyl trifluoroacetate or bis-(p-
nitrophenyl)carbonate, and removal of the Boc
protecting group. The resulting reactive monomer is
polymerized, in the presence of a base such as
triethylamine or N-methylmorpholine as necessary, to

35   give a polypeptide containing hexamer units.

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Copolymerization with a reactive penta- or tetrapeptide
as described herein gives the final bioelastomer of
controlled elasticity. Alternately, intermediate
hexapeptide segments can be independently synthesized
5   and incorporated into a block copolymer also containing
elastomeric units as described below. A blocking
group, such as H-Val-OMe may be added at the conclusion
of the polymerization reaction to convert the remaining
reactive p-nitrophenyl esters to non-reactive terminal
10   groups if desired.

        The elasticity-modifying hexapeptide segments are
believed to operate much in the same way as the hard
segments in segmented polyurethanes. For example, when
the polyhexapeptide, specifically $(VAPGVG)_n$, is
15   dissolved in water at 4°C and the temperature is
raised, aggregation occurs over a relatively narrow
temperature range and that range shifts to lower
temperatures as the concentration is raised. The
aggregation of the polyhexapeptide, in contrast to that
20   of the polypentapeptide, is irreversible in water. The
aggregates can be redissolved in trifluoroethanol-water
mixtures and lyophilyzed to regain water solubility;
i.e., the heat-illicited aggregation of the poly-
hexapeptide is not a true irreversible process. This is
25   thought to be due to the more rigid structure of the
polyhexapeptide wherein on association there is an
interlocking of hydrophobic ridges. Thus, in a
copolymer comprising repeating hexapeptides and
repeating pentapeptides, it appears that the
30   hexapeptide repeats tend to associate selectively
(i.e., to cluster) and that these clusters of stiffer
hexapeptide units, separated by softer polypentapeptide
or polytetrapeptide segments, impart additional
rigidity to the composite material and result in an
35   increased modulus of elasticity and increased tensile

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strength. This is quite analogous to the hard and soft
segments of segmented polyurethanes, such as are
described in Ulrich et al, in Synthetic Biomedical
Polymers: Concepts and Applications, Szycher and
5   Robinson, Eds. Technomic Publishing Co., Inc., West
Port, Connecticut, 29-38 (1980).

Investigations have demonstrated that
polyhexapeptide-polyhexapeptide interactions can occur
within a predominantly polypentapeptide matrix. This
10   is demonstrated by an increased elastic modulus which
is consistent with a hard-segment role for the
synthetic elastomeric polypeptide material. The
results are also consistent with an interlocking by
means of interdigitating hydrophobic ridges of
15   repeating hexapeptide units. Preferred materials
containing the hexameric segments of the invention
would have the general formula $(E_m H_n)_p$, in which E
represents an elastomeric tetrapeptide or pentapeptide
unit and H represents a hexapeptide unit. Particularly
20   preferred are polymers in which m is an integer from 1
to 100 (preferably 3 to 25), n is 3 or greater
(preferably 5 to 10 and $\leq$ m), and p is of such a
magnitude to provide a single polypeptide sequence
having 50-5000 residues, preferably 500-2000 residues
25   (preferably p = 10 to 100). It should be noted that
the formula $(E_m H_n)_p$ does not necessarily represent a
block copolymer, unless so specified, but can also
represent a random copolymer having the indicated
ratios of repeating units, wherein groups of at least
30   three sequential hexamers are present in a random
fashion. Typically, the ratio of elastomer unit to
hexameric unit is from 1:1 to 10:1.

Biocompatibility tests have been conducted in
rabbits on the polypentapeptide and on γ-irradiation
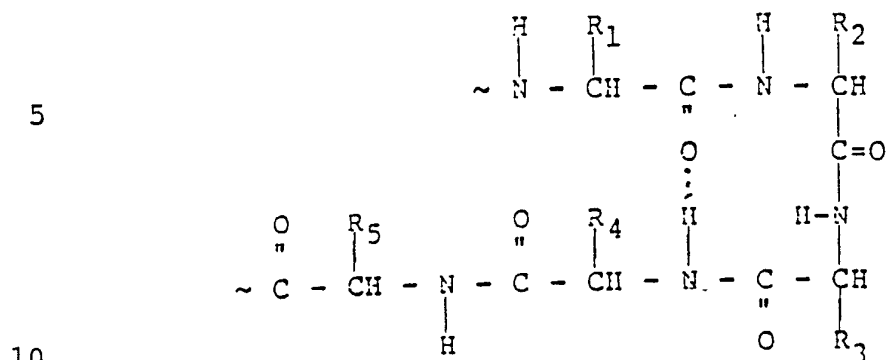35   cross-linked polypentapeptide. The material was found

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to be biocompatible and to be biodegradable. Because
of the similarity of the polyhexapeptide sequences, a
material incorporating hexapeptide sequences as
described herein should have these same

5   characteristics.

    The amount of hexapeptide incorporated into a
polypentapeptide or polytetrapeptide elastomer of the
invention will naturally vary according to the desired
modulus of elasticity and tensile strength.

10   Incorporation of a small relative amount of the
hexapeptide will result in a small increase in the
elastic modulus and tensile strength while introduction
of a larger proportion will result in a larger
increase. Accordingly, the amount necessary to produce

15   any increase in elastic modulus can readily be
determined by simple experimentation. However, in
order to retain sufficient elasticity of the resulting
copolymer for biomedical applications, the ratios
previously described are preferred.

20   The elastomeric units used with the hexapeptide
segments to form the complete bioelastomer have been
described above, including the U.S. patents and
copending applications incorporated by reference. An
essential feature of the elastomeric pentapeptide,

25   tetrapeptide, and D-amino-acid-containing tetra- and
pentapeptide elastomeric units of the earlier
inventions, such as D-Ala^3-containing sequences, is the
existence of a sequence of regularly recurring β-turns
which are characterized by a ten atom hydrogen bonded

30   ring of the following formula:

35

$$\begin{array}{cccc}
\overset{H}{\underset{|}{N}} & \overset{R_1}{\underset{|}{CH}} & \overset{H}{\underset{|}{N}} & \overset{R_2}{\underset{|}{CH}} \\
\sim N - CH - & \underset{\underset{O}{\|}}{C} - & N - & CH \\
& & & \underset{\underset{C=O}{|}}{} \\
\end{array}$$

$$\begin{array}{cccccc}
\overset{O}{\underset{\|}{}} & \overset{R_5}{\underset{|}{}} & \overset{O}{\underset{\|}{}} & \overset{R_4}{\underset{|}{}} & \overset{H}{\underset{\|}{}} & \overset{H-N}{\underset{|}{}} \\
\sim C - & CH - & N - & C - & CH - & N.- & C - & CH \\
& & \underset{H}{|} & & & & \underset{O}{\|} & \underset{R_3}{|} \\
\end{array}$$

In this formula, R_1 through R_5 represent the side groups of the respective amino acid residues.

The spiral structures produced by a series of β-turns are more open than the more common α-helix. As a result, the atoms between the atoms that participate in hydrogen bonding have a relatively greater freedom of movement, more so than in an α-helix. This is particularly true of librational motions involving non-hydrogen bonded peptide moieties in the β-spiral. A libration is a torsional oscillation involving simultaneous rotational motions of the two single bonds on each side of a librating moiety. The moiety involved in a libration may be a single peptide bond or several peptide residues. For adequate freedom of motion to exist, it is important, however, that the carbonyl oxygen and the amino hydrogen of the peptide bond not be involved in a motional restricting hydrogen bonding to other parts of the molecule or to other peptide molecules. Otherwise a greater energy barrier to the libration exists and motion will be restricted. Since non-hydrogen-bonded segments having freedom of motion exist in the β-spiral between the points of hydrogen bonding for the β-turns, these

segments may be said to be librationally suspended with
librational capabilities. Librationally active
suspended segments therefore are a structural feature
that exists in the elastomeric polymer because of the
5   repeating β-turns with relatively infrequent hydrogen
bonding.

Another factor leading to the high librational
freedom of such molecules is the absence of polar
interactions between the amino acid residues, either
10   intrachain or interchain, other than the previously
mentioned hydrogen bond. The amino acid residues
present are generally all hydrophobic or glycine and
accordingly do not exert significant forces on one
another through space. If charged or polar groups were
15   present, electrostatic interactions could limit
librational freedom and restrict the number of
available states in the relaxed (non-extended) form of
the molecules and which would shift the temperature of
the inverse temperature. Polar and charged amino acid
20   residues are not strictly prohibited, and moreover may
occur when incorporated in cell attachment sequences
and chemotactic peptides, if their presence does not
destroy the elasticity of the polypeptide molecule as a
whole. For example, an occasional serine residue is
25   present in the polypentapeptide sequence of naturally
occurring porcine tropoelastin without destroying
elasticity. Accordingly, hydrophobic amino acid
residues and glycine are preferred in forming
elastomeric polypeptides of the present type although
30   other amino acids may be present to a small extent.

The size of the repeating unit of the elastomeric
component is important in determining the stability and
dynamic properties of the β-spiral. Repeating units
having fewer than four or more than five amino acid
35   residues can form β-spirals having the required

librational motions when using a polynona-sequence
comprised of a pentamer and a tetramer. Thus,
elastomers of the present type appear to be limited to
polypeptides having tetrapeptide or pentapeptide
5   repeating units in the elastomeric component or perhaps
having a regular combination of pentamer plus tetramer
to afford a polynonapeptide. Elastomers containing an
amino acid residue of opposite chirality at position
three, as disclosed in U.S. Patent 4,500,700 and
10   discussed briefly below, are also believed to be
limited to polypentapeptides or polytetrapeptides, with
polypentapeptides being particularly important.

        Selective replacement of glycine residues at
position 3 in the elastomeric repeating units with
15   hydrophobic D-residues gives an elastomer having a
higher modulus of elasticity. Studies of the dominant
conformational feature of the polypentapeptide of
elastin, the Type II Pro_2-Gly_3 β-turn previously
discussed, indicate that a D-residue at position three
20   will stabilize the β-turn. Substituting a D-amino acid
residue for the Gly_3 residue produces an elastomeric
molecule (after cross-linking) having an elastic
(Young's) modulus approximately twice that obtained for
the corresponding molecule having a Gly_3 residue.

25   When a repeating unit having an amino acid residue
of opposite chirality is utilized, it is preferred that
the amino acid residue in position three be a
hydrophobic D-amino acid although other D-amino acids
are also contemplated to be within the scope of the
30   present materials. Amino acid residues having no more
than 10 carbon atoms in their amino acid side chain are
preferred. Preferred hydrophobic side chains are
alkyl, aryl, and arylalkyl, where aryl represents a
phenyl or alkyl-substituted phenyl group. Particularly
35   preferred are the residues of D-alanine, D-valine, D-

leucine, D-isoleucine, D-phenylalanine, D-2-
aminobutanoic acid, and other molecules of similar
size, polarity, and chirality. Especially preferred
are alkyl side chains having 1-5 carbon atoms in an α-
5   amino acid residue having an α-hydrogen.

The choice of individual amino acids from which to
synthesize the remaining sections of the elastomeric
repeating units and resulting polypeptide is
unrestricted so long as the resulting structure
10 comprises librationally suspended segments in a
spiral. The amino acids are not restricted to α-amino
acids, although these are preferred, since it has
recently become possible to predict the occurrence of
β-turns from the α-amino acid sequence of a
15 polypeptide. A review article discussing the
prediction of protein conformation, including the
prediction of β-turns, was published by Chou and
Fasman, Ann. Rev. Biochem., 47, 251 (1978), which is
herein incorporated by reference. The size of the side
20 chains present in the hydrophobic amino acids does not
greatly affect the β-spiral since the side chains with
some important but non-restrictive interturn
hydrophobic interactions. However, in order to
minimize interchain interactions, it is preferred that
25 the side chain contain no more than 10 carbon atoms.
Preferred hydrophobic side chains are the same as those
previously described for position three. In addition,
it appears from the studies leading to the present
invention that preferred side chains of the amino acids
30 are hydrogen or hydrocarbon chains having 1-7 carbon
atoms. Examples of especially preferred residues are
glycine and the naturally occuring L-amino acids
alanine, valine, leucine, and isoleucine as well as
closely related molecules such as 2-methyl-
35 2-aminopropanoic acid, L-2-aminobutanoic acid, and

L-2-methyl-2-aminobutanoic acid, although it is
preferred that the α-carbon have an α-hydrogen.
Proline is also a preferred amino acid.

Given positions of the repeating units of the
5   elastomeric component have amino acid residues that are
particularly preferred. The first amino acid residue
is preferred to be valine, leucine, or isoleucine: the
second is preferred to be proline; the third has been
previously discussed; the fourth is preferred to be
10   valine, leucine or isoleucine; and the fifth residue is
preferred to be glycine.

An elastomeric component consisting entirely of
repeating units in which the third amino acid residue
is of opposite chirality, as described herein, has an
15   elastic modulus approximately twice that of an
otherwise identical polypeptide in which all the amino
acids have the same chirality, such as those described
in U.S. Patent 4,187,852. Accordingly, it is possible
to easily alter the elastic modulus by using a mixture
20   of monomers and controlling the amount of crosslinking
between adjacent peptide chains. Elastic modulus is
proportional to the number of crosslinks, although not
in a strictly linear fashion. It should be noted that
the modification of elastic modulus described above by
25   using amino acid residues of opposite chirality
operates by an entirely different mechanism from that
of the present materials.

Because of the chirality of amino acids and of
polypeptides produced therefrom, an equally effective
30   polypeptide can be produced by using polypentapeptide
repeating units in which all of the amino acids having
chiral centers are of the opposite chirality from that
previously described: i.e., L-amino acid residues are
replaced with D-amino acids. Since both L- and D-amino
35   acids are available commercially and can be used as

starting materials in a synthesis of the polypeptide of
the invention, for example in the method disclosed
below, either of these species of the invention may be
easily produced. However, since D-amino acids are
5   relatively more expensive, the more preferred species
is that in which all or most of the amino acid residues
of the elastomeric component are derived from L-α-amino
acids and only the residue at position 3 is derived
from a D-amino acid.

10   Methods of preparing elastomeric components in
which the third position is occupied by a glycine
residue have been disclosed in Rapaka and Urry, Int. J.
Peptide Protein Res., 11, 97 (1978), Urry et al,
Biochemistry, 13, 609 (1974), and Urry et al, J. Mol.
15 Biol., 96, 101 (1975), which are herein incorporated by
reference. The synthesis of these peptides is
straightforward and can be easily modified to allow
production of any polymer described in this
application, if desired. The following summary, which
20 is not to be considered limiting, is an example of one
general method of synthesizing the polypeptides.

The first step in the formation of the elastomeric
copolymer of the invention usually is synthesis of the
various peptide monomers. Any of the classical methods
25 of producing peptide molecules may be used in
synthesizing the building blocks of the polymers of the
present materials. For example, synthesis can be
carried out by classical solution techniques starting
from the C-terminal amino acid as a benzyl ester p-
30 tosylate. Each successive amino acid is then coupled
to the growing peptide chain by means of a watersoluble
carbodiimide and 1-hydroxybenzotriazole. A typically
used carbodiimide is 1-(3-dimethylaminopropyl)-3-
ethylcarbodiimide hydrochloride (EDCI). During the
35 coupling reaction the amino group is protected. The

protecting group is then removed after condensation has
taken place. A suitable protecting group is tert-
butyloxycarbonyl (Boc), which can easily be removed by
trifluoroacetic acid.

5   The structure, examples of synthesis, and use of
these elastomeric compounds and of various compositions
containing these compounds is fully disclosed in the
incorporated patent applications. The present material
is not related to these elastomeric components

10   themselves but uses them in the formation of the total
elastomeric material containing the tetrameric,
pentameric and hexameric segments for the prevention of
adhesion.

       Synthesis of the final elastomeric polypeptides

15   containing the elastomer component and elasticity-
modifying hexapeptide component of the present
invention is straightforward and easily accomplished by
a peptide chemist. See, for example, the techniques
described in Li and Yamashiro, J. Amer. Chem. Soc., 92,

20   7608-7609 (1970) which is herein incorporated by
reference. The resulting generally polypeptides have
the structure $X-[(\text{elastomeric unit})_m (\text{hexameric unit})_n]_p-Y$
where X and Y represent any chemically compatible
end group on the amino and carboxyl ends of the

25   molecule, respectively, and m, n, and p are as
previously described. Block copolymers are preferred,
although random copolymers are also suitable. Block
copolymers can be synthesized sequentially in an
automatic peptide synthesizer or by reacting preformed

30   units consisting of activated elastomeric and
hexapeptide units (which is preferred) or even by
microbial fermentation. If the reaction with preformed
units is used, it is preferred to use a shear stirring
technique to orient the linear elastomeric units and to

35   use EDCI as an activator. Relatively long reaction

times and replenishment of EDCI during the course of
reaction are preferred. Particularly preferred are
polypeptides having molecular weights greater than
10,000 daltons, even greater than 100,000 daltons. It
5   is possible that one or more amino acid residue or
segment of amino acid residues (such as the
crosslinking segments later discussed) may be
interspersed within the polypeptide chain so long as
the elasticity and biocompatibility of the resulting
10   molecule is not completely disrupted. Examples of
terminal end groups that can be present in
bioelastomers of the invention include the repeating
peptide units themselves with free amino or carboxylic
acid groups or salts thereof and peptide or amino acid
15   units that have retained a blocking group that was
present during synthesis of the polypeptide or that
have a blocking group added after formation of the   ·
polypeptide. Examples of blocking groups include t-
butyloxycarbonyl, formyl, and acetyl for the amino end
20   of the molecule and esters, such as methyl esters, as
well as amides, such as the amides of ammonia and
methyl amine, for the acid end of the molecule. The
end groups are not critical and can be any organic or
inorganic group that does not destroy the β-turn
25   conformation of the elastomeric component and does not
negate biocomptability and that allows a suitable rate
of degradation for the molecule as a whole.

        It is preferred that a bioelastomer containing
elastomeric and hexapeptide repeating units be heated
30   in order to kinetically drive the molecules to form
hexamer-hexamer interaction. By heating the material,
the hexamer segments are·driven together in   .
interlocking as described above. When the products are
cooled, insufficient kinetic energy exists to unlock
35   the interlocked hexamer-hexamer segments.

The temperature to which the sample is raised in order to effect this heat treatment can readily be determined for a particular copolymer in at least two ways. First, elastic modulus can be measured as a function of temperature in a small sample of the cross-linked copolymer product. There is a rapid increase in modulus of elasticity at the temperature at which the hexamer-hexamer interactions begin to occur. Alternatively, turbidity measurements provide the same information. Opacity of the samples indicates interlocking of the hexamer regions. The bioelastomers of the invention are quite stable to heat treatment. For example, a polypentapeptide (lacking the hexamers of the present invention) can be heated for 24 hours at 80°C without significant degradation. The polyhexapeptide is even more stable to heat degradation. The bioelastomers of the invention can be autoclaved with retention of desirable elasticity, thereby sterilizing the bioelastomer while providing the increased modulus of elasticity and tensile strength at the same time.

When bioelastomers of the invention are heat treated, it is preferred that this treatment take place prior to any of the cross-linking processes described later.

In addition to having an increased modulus of elasticity, bioelastomers incorporating PHP also show an increased tensile strength and thus a greater resistance to tearing than bioelastomers prepared solely from the elastomeric units described in this specification.

An improved prosthetic material of higher tensile strength can be produced by using a collagen-like load bearing component in addition to an elastomeric component of the correct elastic modulus. This can be

achieved by compounding the synthetic elastomeric high
polymers described above to a second material with
greater strength. The second material forms the core
of the composite fiber and will be referred to as the
5   "collagen analogue" or "core fiber". The term core
fiber is not limited to those forms of elastomeric
composite materials in which a first fiber is coated
with a second material, but also refers to other forms
in which a strength giving fiber (the core fiber) is
10   chemically bonded to a second component that is
elastomeric (the polypeptide). For example,
elastomeric polypeptide fibers may form strands between
the segments of a crimped core fiber. The essential
feature is that a chemical bond (of any type) exists
15   between the surface of the core fiber and the
elastomeric polypeptide so that the two components do
not become separated while the elastomeric component is
being stretched or is reforming the relaxed ß-spiral.
The chemical bond may be covalent or ionic bonding,
20   hydrogen bonding, or the result of electrostatic
interactions of various types, such as ion-dipole and
diopole-dipole interactions. Covalent bonding is
preferred. Linkages may be formed in any conventional
manner and, if covalent honds are to be formed, they
25   can be accomplished by reacting a functional group of
the polypeptide with a functional group of the core
fiber. The functional groups may be present naturally
as part of the polypeptide or core fiber or may be
formed later, for example, by suitable chemical
30   reactions involving the already formed core fiber or
polypeptide. Composite materials of this type are
fully described in U.S. Patent 4,474,851, which is
herein incorporated by reference.
      It is generally desirable to cross-link the
35   molecules of the polypeptide prior to use in vivo in

order to increase its strength and elasticity. If a
composite fiber is being formed, it is preferred to
perform the cross-linking after the polypeptide has
adhered to the core fiber. The method of creating the
5   linkage is not limited to the methods disclosed in this
application and may be any method of covalent or non-
covalent linkage that does not prevent the elastomeric
copolymer or the composite fiber from behaving as an
elastomer. Suitable methods and types of linkages
10   include cross-linking with ionizing irradiation and
chemical modification or substitution of amino acid
residues of the peptide repeating units and of the
collagen analogue repeating units in order to form
reactive side groups that undergo chemical reaction
15   with each other (chemical cross-linking) e.g., by amide
linkage, aldol condensation, Schiff base formation,
enzymatic cross-linking by lysyl oxidase, or ester
formation or even disulfide bridge formation. Another
suitable method of cross-linking comprises the use of
20   photoactivated agents such as those giving rise to
carbenes or nitrenes which may be attached as amino
acid side groups or introduced as separate diffusible
molecules.

A particular type of chemical cross-linking occurs
25   when polypeptides are prepared in which some of the
repeating units are replaced by units in which one of
the amino acid residues is replaced by the residue of
an amino acid that has a reactive side chain. For
example, it is possible to prepare a first batch of
30   polypeptide in which a residue of some of the repeating
units is replaced by an amino dicarboxylic acid, such
as aspartic or glutamic acid, and a second batch of.
polypeptide in which a residue of some of the repeating
units is replaced by a diamino carboxylic acid, such as
35   lysine or ornithine. After a mixture of these two

batches has been formed into a sheath around the core
fiber, the free amino and carboxylic acid side group
are allowed to react to create the cross-linkages.
Formation of cross-linked polypentapeptide produced in
5   this manner is described in U.S. Patent 4,187,852,
which is herein incorporated by reference. If chemical
cross-linking is used, it is also necessary to provide
reactive functional groups in the core fiber so that
linkages between the peptide elastomer and the core
10   fiber will also occur. Such modifications are well
understood by polymer chemists and may include, for
example, glycidyl esters of acrylates or methacrylates
(as examples of reactive groups present during
formation of the core polymer), or amino or carboxylic
15   acid groups added to the terephthalic acid moiety of
Dacron (as examples of reactive groups formed after
formation of the core fiber).

The degree of cross-linking is such that
elastomeric properties are imparted to the resulting
20   composite fiber and can be varied to provide the
desired dynamic mechanical properties. Preferred is an
average of one cross-link for every 5 to 100 elastomer
repeating units with 10 to 50 being most preferred. The
degree of chemical cross-linking can be controlled by
25   selecting the proper proportions of reagents. In
general, the ratio of repeating units with reactive
side groups to unmodified repeating units within a
single molecule can vary from 1:1 to 1:20 with a ratio
of about 1:5 being preferred. When two batches of
30   polypeptide containing carboxylate or amino side groups
as described above are used, the ratio of
carboxylate-side-group-containing polypeptide to amino-
side-group-containing polypeptide can vary from 4:1 to
1:4 with a ratio of about 1:1 being preferred.

35

An additional method of chemical cross-linking
using cross-linking units capable of being cross-linked
by lysyl oxidase is described in U.S. Patent 4,589,882.

When irradiation cross-linking is performed, a
5   satisfactory approach is irradiation with gamma
radiation from a cobalt-60 source. Other radiation
energies required to provide a cross-linking action
without excessive destruction of the core fiber or
elastomeric peptide structure may be easily determined
10   by simple experimentation. The degree of cross-linking
is determined by the length of time and energy of the
irradiation when irradiation cross-linking is
performed. At least two cross-linkages per molecule
are needed. The number of cross-linkages per molecule
15   and the elastic modulus increase as the radiation dose
increases. The requisite time for any desired amount
of cross-linking is easily determined by simple
experimentation for any given source of irradiation
energy. Samples of non-crosslinked polymer or
20   composite fiber are exposed to the source of ionizing
energy for varying lengths of time, and the resulting
elastic modulus is measured. In this manner the
irradiation time required to produce an elastic modulus
necessary to match a specific design characteristic of
25   the polymer or composite fiber can easily be
determined.

With respect to the doses of γ-irradiation
required for cross-linking, in general, the lowest
possible dose should be used as is needed to match the
compliance of the vascular wall. The dosage will vary
30   depending upon the constituents of the basic sequential
,polypeptide. However, in most instances,
the γ-irradiation dose is not in excess of about 30-
40 M rad.

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The materials of this invention, as noted, are advantageously used as materials for vascular prostheses.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

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Example

Abbreviations: BOc, tert-butyloxycarbonyl; Bzl,
benzyl; DMSO, dimethylsulfoxide; EDCI, 1-(3-
dimethylaminopropyl)-3-ethylcarbodiimide; HOBt, 1-
hydroxybenzotriazole; IBCF, isobutylchloroformate:
NMM, n-methyl morpholine; ONp, p-nitrophenylester; TFA,
trifluoracetic acid; A, alanine; G, glycine; V, valine;
P, proline; PPP, polypentapeptide (VPGVG)n; PHP,
polyhexapeptide (VAPGVG)n and P(HP)P, cosequential
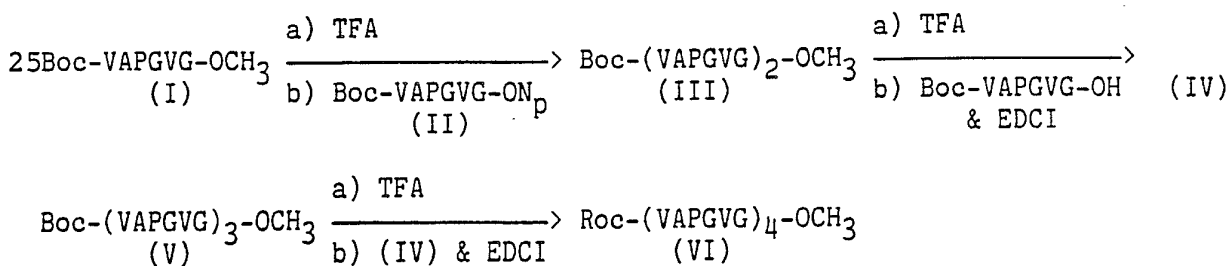polyhexapentapeptide.

EXPERIMENTAL DETAILS
Peptide Synthesis

The synthesis of the sequential polypeptides of
the hexapeptide sequence, Boc-(VAPGVG)$_n$-OCH$_3$ where n =
2, 3, and 4 was carried out by classical solution
methods as shown in Scheme III below:

Scheme III

Synthesis of the Sequential Polypeptides of
the Hexamer Sequence

$$\text{Boc-VAPGVG-OCH}_3 \xrightarrow[\text{b) Boc-VAPGVG-ON}_p]{\text{a) TFA}} \text{Boc-(VAPGVG)}_2\text{-OCH}_3 \xrightarrow[\substack{\text{b) Boc-VAPGVG-OH} \\ \text{\& EDCI}}]{\text{a) TFA}} \text{(IV)}$$

$$(I) \qquad (II) \qquad (III)$$

$$\text{Boc-(VAPGVG)}_3\text{-OCH}_3 \xrightarrow[\text{b) (IV) \& EDCI}]{\text{a) TFA}} \text{Boc-(VAPGVG)}_4\text{-OCH}_3$$

$$(V) \qquad\qquad (VI)$$

Boc-VAPGVG-OCH3 (I) prepared as described in Urry
et al., Int. J. Pept. Protein Res., 7, 367-378 (1975),
was deblocked with TFA and coupled with Boc-VAPGVG-ONp
(II) in the presence of HOBt to obtain Boc-

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(VAPGVG)$_2$OCH$_3$ (III). After deblocking III, it was coupled with Boc-VAPGVG-OH (IV) using the water soluble carbodiimide, EDCI, to obtain the next higher homologue

5   V. Similarly Boc-(VAPGVG)$_4$-OCH$_3$ (VI) was also obtained. The syntheses and purity of the oligohexapeptides with n = 2 and 3 have been verified by C-13 NMR.

The synthesis of the polyhexapeptide, H-(VAPGVG)$_n$V-OCH$_3$ has been carried out starting from the

10 monomer sequence H-VAPGVG-ONp. Recently, it was observed in the case of the preparation of the PPP, that with the permutation where Pro is present as the C-terminal amino acid in the monomer sequence, as HGVGVP-ONp instead of H-VPGVG-ONp, a very high

15 molecular weight polymer (about 100,000 dalton) could be obtained Urry et al., in Biocompatibility of Natural Tissues and Their Synthetic Analogues (D.F. Williams, Ed.) CRC Press, Inc., Boca Raton, Florida, 89-116 (1985). Hence a similar approach was taken for the

20 preparation of the polyhexapeptide with Pro as the C-terminal amino acid in the monomer sequence. The synthesis of poly(GVGVAP)-OH is shown in Scheme IV. The product may, of course, also be written H-GVG(VAPGVG)$_n$VAP-OH.
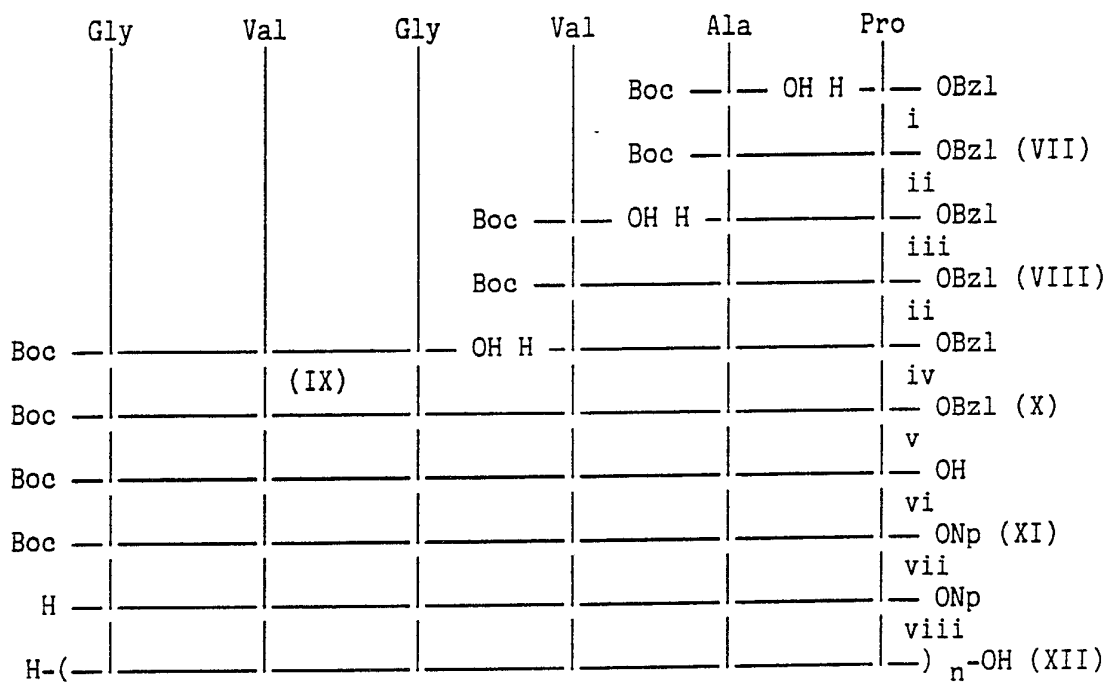
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Scheme IV

Synthesis of H-(GVGVAP)$_n$-OH

```
        Gly      Val      Gly      Val      Ala      Pro
                                         Boc —!— OH H —!— OBzl
                                                       | i
                                         Boc —!_____|— OBzl (VII)
                                                       | ii
                               Boc —!— OH H —!_____|— OBzl
                                                       | iii
                               Boc —!_____|_____|— OBzl (VIII)
                                                       | ii
Boc —!_____|_____|— OH H —!_____|_____|— OBzl
     |        | (IX)   |        |        |        | iv
Boc —!_____|_____|_____|_____|_____|— OBzl (X)
     |        |        |        |        |        | v
Boc —!_____|_____|_____|_____|_____|— OH
     |        |        |        |        |        | vi
Boc —!_____|_____|_____|_____|_____|— ONp (XI)
     |        |        |        |        |        | vii
  H —!_____|_____|_____|_____|_____|— ONp
     |        |        |        |        |        | viii
H-(—!_____|_____|_____|_____|_____|—)$_n$-OH (XII)
```

i) IBCF-HOBt; ii) HCl/Dioxane; iii) IBCF;
iv) EDCI-HOBt; v) H$_2$-Pd/C; vi) Bis-(p-nitrophenyl)
carbonate; vii) TFA; viii) DMSO-NMM

Boc-AP-OBzl (VII) was prepared by the mixed
anhydride method in the presence of HOBt. After
deblocking, the coupling was carried out with Boc-Val-
OH to obtain the tripeptide benzyl ester (VIII) which
5   was deblocked and coupled with Boc-GVG-OH (IX) to
obtain the hexapeptide, X. Following hydrogenolysis,
the peptide acid was converted to the p-nitrophenyl
ester (XI) using bis(p-nitrophenyl) carbonate. After
removal of the Boc group with TFA, a one-molar solution
10   of the active ester in DMSO was polymerized for 14 days
in the presence of 1.6 equiv. of NMM. On diluting with
water the PHP was dialyzed against water, first using
3,500 molecular weight cut-off dialysis tubing for 7
days and then 50,000 M.Wt. cut-off tubing for 7 more
15   days and finally the retentate was lyophilized.
      Random copolymerization of hexa and
pentapeptides: The active p-nitrophenylesters of the
hexapeptide, H-GVGVAP-ONp, and the pentapeptide,
HGVGVP-ONp, were mixed in the desired ratio (1:2) and
20   polymerized in the usual way. The product is a
sequential polypeptide in which in a single chain there
is presumably a random mixture of hexapeptides and
pentapeptides in a 1:2 ratio. This product will be
called a cosequential polyhexapentapeptide and will be
25   abbreviated as P(HP)P. The purity of the intermediate
and final compounds was checked by thin layer
chromatography, elemental analyses and nuclear magnetic
resonance. Synthesis and purity of the
polyhexapeptide, PHP, the cosequential
30   polyhexapentapeptide, P(HP)P, and for comparison of the
polypentapeptide, PPP, have been verified by C-13 NMR.
      Temperature profiles of aggregation: Temperature
profiles of aggregation (turbidity profiles) were
obtained with a Cary 14 spectrophotometer set at 300 nm
35   to follow light scattering as a function of increasing

temperature. The heating rate was 30°C per hour and to
insure appropriate mixing of the polymers the sample
cell was equipped with a 20 KHz vibrator. Samples were
run in duplicate with fresh material used each time in
5   a 1 mm rectangular cell with a total volume of 0.3 ml.

Reversibility of the polypeptides was checked
immediately after each temperature run by exposing the
sample to an ice bath for 30 minutes. If the
aggregation was not reversible, the sample was further
10   incubated at 4°C for 16 hours. For the
oligohexapeptides, reversibility was checked after the
sample had been taken to 72°C where n = 3 and to 60°C
where n = 4 and then when both had been taken to 80°C.

Preparation of polyhexapeptide sheet from
15   trifluoroethanol: The polyhexapeptide (PHP) was
evaporated from trifluoroethanol (TFE) in a round
bottom flask after which ether was added, and the
resulting PHP sheet was removed. This material was cut
into strips for the stress-strain studies.

20   Preparation of the γ-irradiation cross-linked
elastomers: A quantity of 120 mg of dry sample was
placed in the bottom of a cryotube to which 204 μl of
distilled water were added. The samples were allowed
to hydrate at 4°C to form a viscoelastic mass. In each
25   tube a Plexiglas pestle was then inserted which had a
1 mm x 10 mm channel turned in it. As the pestle was
inserted, the viscoelastic sample flowed into and
filled the channel to form a continuous band with
dimensions of 28 mm x 10 mm x 1 mm. The P(HP)P samples
30   within the channel were held at 65°C for 17 hours with
slow rotation of the pestle. The samples were then
placed in dry ice and taken to the γ-irradiation
source. Irradiation cross-linking of the samples was
carried out at the Auburn University Nuclear Science
35   Center. The samples were placed at the center of a

group of cobalt sources arranged in concentric
circles. The configuration gave approximately 0.45
MRAD per hour gamma irradiation. The samples were
exposed for 44.19 hours to give a total of 20 MRADs.
5   The elastomeric bands are referred to as X^{20}-PPP for
the cross-linked polypentapeptide, X^{20}-(PHP+PPP) for
the cross-linked 1:2 mixture, respectively, of
polyhexapeptide and polypentapeptide and X^{20}-P(HP)P for
the cosequential polyhexapentapeptide with a random
10   mixture of hexapeptide to pentapeptide (1:2) in the
original polymerization.

Stress-strain studies: A strip of the
polyhexapeptide sheet measuring 24 mm x 4.64 mm x 0.28
mm was clamped in the stress-strain apparatus with the
15   distance between the grips being 10 mm. Elongation of
the sample was done at a rate of 1.1 mm/min. The X-
axis of the plotter recording the displacement of the
moving grip was calibrated to 25.4 mm pen movement for
a 0.1 mm grip displacement. The Statham UC-2
20   transducer with a UL4-2 load cell accessory used in our
apparatus has a sensing element displacement of 2.15 x
10^{-4} mm/gram of applied force. This correction was
applied in calculating the elastic (Young's) modulus.
The cross-section of each of the γ-irradiation
25   crosslinked samples was measured prior to and after
completion of each run. The elastic modulus of each
sample was measured at 40°C in water. The cross-linked
cosequential polypeptide, X^{20}-P(HP)P, samples were then
allowed to set for 12 hours at 65°C and again the
30   elastic modulus was measured at 40°C. The X^{20}-P(HP)P
samples were subsequently swollen at 20°C in water for
five hours, heated at 80°C in water for 17 hours and
then the elastic moduli were again determined. During
γ-irradiation cross-linking of some samples, vacuoles
35   formed. When elastic moduli were determined no

correction was made for the crosssectional area of the
vacuoles such that the elastic moduli are minimal
values and can generally be considered to be about
twice the value reported for the X^{20}-PPP and X^{20}-P(HP)P
5   samples. The important element for the latter sample
is the increase in elastic modulus on heat treatment at
65°C and 80°C.

RESULTS

Aggregational Properties of Oligohexapeptides
10   When the hexapeptide (n = 1) and the dodecapeptide
(n = 2) were examined, they exhibited little if any
aggregation on raising the temperature to 80°C. When
the octadecapeptide (n = 3) was examined, however, it
gave a temperature profile for turbidity with a
15 midpoint just above 70°C, thereby demonstrating
aggregation of polypeptide chains. On immediately
lowering the temperature the aggregates redissolved,
but if the temperature was raised to 80°C and held
there for a short period, the association in water did
20 not reverse on standing overnight at 4°C. When the
tetraeicosapeptide (n = 4) was examined, the midpoint
for the turbidity profile was shifted to a lower
temperature, 57°C. This aggregation was found to be
irreversible in water. Thus for the oligohexapeptides
25 a repeating sequence of three to four hexapeptides is
required before the polyhexapeptide-polyhexapeptide
interaction is irreversible. To achieve
irreversibility with heating at temperatures less than
80°C an n of four is required.
30   The turbidity profiles for high molecular weight
polyhexapeptide (n = 100) were determined. The
polyhexapeptide-polyhexapeptide interaction was
irreversible in water, and dissolution in
trifluoroethanol and lyophilization with increasing
35

amounts of water is required to again attain solubility
in water at low temperatures. The turbidity profiles
for a series of concentrations of high molecular weight
polypentapeptide (n = 100) were also determined.

5   Aggregation was immediately reversible on lowering the
temperature. In an initial test as to whether
hexapeptide sequences might function in an interlocking
role when randomly interspersed in a polypeptide chain
comprised of mostly pentapeptide, the random

10   cosequential polyhexapentapeptide, P(HP)P, was prepared
as outlined in the Experimental Section. The turbidity
profiles for P(HP)P as a function of concentration were
also determined. The aggregates, formed by heating
this random copolymer of hexapeptide and pentapeptide

15   with a ratio of 1:2, respectively, immediately
redissolved on lowering the temperature below 25°C. If
there are in the P(HP)P sufficiently long runs of
uninterrupted repeating hexapeptides to effect some
interlocking at elevated tempecatures, these

20   associations are separated by the hydration (swelling)
of pentamer sequences on lowering the temperature.
Whether such associations might be present at 40°C can
be tested in the cross-linked samples by the
stress-strain studies reported below.

25   When a lower molecular weight polyhexapeptide with
n = 40 is placed in one chamber of a tandem cell and
polypentapeptide in the other, delayed turbitity was
seen as the temperature was increased. The delayed
turbidity change is believed to be due to aggregation

30   of the lower molecular weight polyhexapeptide. On
mixing the separately aggregated PHP and PPP, the PHP
aggregation is not reversed on standing at 4°C for more
than 12 hours. If however the lower molecular weight
PHP and the PPP are mixed at low temperature as

35   solutions, on immediately lowering the temperature all

aggregates redissolve. This indicates that there is
significant PHP-PPP association on raising the
temperature. When higher molecular weight PHP (n =
100) and PPP (n = 100) are combined as solutions at low

5   temperature, a mixed aggregation is seen. This mixed
aggregation does not redissolve on standing at 4°C for
several days. This demonstrates that there also can
occur sufficient PHP-PHP interaction in the presence of
PPP to achieve irreversibility i.e. interlocking.

10

Stress-Strain Studies

With 20 MRAD γ-irradiation of polypentapeptide
carried out in the same manner as with the hexapeptide
containing samples, the elastic modulus of the
crosslinked polypentapeptide, X^{20}-PPP, was in the range

15   of 2 to 3 x 10^5 dynes/cm^2 with the measurement taking
place in water at 40°C. A stress-strain curve can be
used to compare polypeptides modified to contain
hexamer units. As a reference for the cellophane-like
polyhexapeptide, a sheet of PHP was cast from a high

20   concentration in trifluoroethanol. The elastic modulus
of this strip was found to be 7 x 10^9 dynes/cm^2. A
representative sample of cosequential
polyhexapentapeptide, cross-linked by 20 MRAD γ-
irradiation, i.e. X^{20}-P(HP)P, gave an initial value of

25   2.2 x 10^5 dynes/cm^2. On heating in water at 65°C for
12 hours and re-examining at 40°C in water, the elastic
modulus had increased to 4.2 x 10^5 dynes/cm^2. The
sample was then kept at 20°C in water for 5 hours and
then heated at 80°C for 17 hours. The resulting

30   elastic modulus at 40°C in water had decreased to 3.3 x
10^5 dynes/cm^2. Averaged values for three samples were
1.9 x 10^5 dynes/cm^2, 4.2 x 10^5 dynes/cm^2 and 3.4 x 10^5
dynes/cm^2; these are for the initial values, the value
after heat treatment at 65°C and the value after

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heating at 80°C, respectively. The rationale for the
choice of temperatures for the heat treatments derives
from the turbidity profiles for the
oligohexapeptides. A temperature of 65°C resulted in
5   irreversible association of oligohexapeptides with n =
4. It would appear from the near doubling of elastic
modulus on heating at 65°C that some association of
hexapeptide units did occur which functioned like
additional crosslinks. Irreversible association of
10   oligohexapeptides with n = 3 did not occur until 80°C
but association of P(HP)P was completely reversed on
going below 25°C (see results of aggregation studies
above). On this basis the X^{20}-P(HP)P, that had been
heated at 65°C, was allowed to swell at 20°C for 5
15   hours. In analogy to the reversible aggregation of
P(HP)P, this is expected to reverse all
hexapeptidehexapeptide interactions. The samples were
then heated at 80°C to see if additional putative
(hexapeptide)$_n$(hexapeptide)$_n$ associations might
20   occur. This treatment caused a decrease in elastic
modulus from what had been obtained after 65°C
treatment. While association of repeating hexapeptides
with n = 3 could possibly have been achieved, it is
known that holding the temperature of PPP coacervate at
25   80°C results in a very slow destructuring of the
polypentapeptide. This and even the heat destructuring
of the conformations of hexapeptide repeats within the
co-sequential polypeptide are possible explanations of
the decrease in elastic modulus resulting from the 80°C
30   treatment.
        Mixing polyhexapeptide with polypentapeptide in a
1:2 ratio produced polymers which were codissolved in a
minimal amount of water (about 60% by weight) at 4°C in
the cryotube. The pestle was then inserted causing the
35   viscoelastic solution to flow into the channel turned

in the pestle. The pestle was rotated with the sample
in an ice bath for several hours and the sample was
then placed in dry ice until γ-irradiation cross-linked
at 20 Mrad. The product can be referred to as
X^{20}-(PHP+PPP). The elastic modulus at between 50 to
60% extension was 2.6 x 10^6 dynes/cm^2. This value is
essentially identical to that of natural fibrous
elastin which similarly exhibits a lower initial
slope. There is a marked reproducible hysteresis which
is a result of the slow rate of retraction of the
sample. The rate of change in length was approximately
1 mm/min.

    In addition to the particular sequences noted
above throughout the present specification, other
sequences of amino acids may be incorporated in the
elastomeric material of the present invention without
limitation provided that the material so made retains
elasticity and exhibits an inverse temperature
transition and the properties associated therewith.

    In particular, and for example, while the
hexameric sequence described above ſAPGVGVƚ, used to
enhance the mechanical properties of a bioelastomer
containing the same, is most often used, other
hexameric sequences may also be used. In the sequence
ſAPGVGVƚ, instead of A, Val, Ile, Phe and Leu may be
used. Instead of P, Val, Ile, Phe, Leu or even Ala may
be used. However, it is preferred that P be proline.
Instead of the first G, Ala may be used. Instead of
the first V, Phe, Leu, Ile or Ala may be used. Instead
of the second V, Phe, Leu, Ile, Val or Ala may be used.
    Notably, occasional substitutions with the full
range of known and available amino acids may be used
with the proviso that the biocompatibility,
biodegradability and elastomeric properties be
maintained as has been disclosed in pending
applications.

Furthermore, in synthesizing the polypeptides of
the present invention, since all of the amino acids
present in the polypeptides of the invention have
corresponding DNA codons, the polypeptides can be

5   produced by genetic engineering using synthetic genes
containing codons that correspond to the desired amino
acid sequence.

B.   THE PHYSICAL PROPERTIES OF THE COMPOSITE MATRIX

10   The basic elastomeric polypeptide is the
polypentapeptide, $(Val-Pro-Gly-Val-Gly)_n$ abbreviated
$(VPGVG)_n$ or simply PPP. A viscoelastic state of the
PPP which is approximately 40% peptide and 60% water by
weight may be γ-irradiation cross-linked to form an

15   elastomeric matrix. The elastic modulus (Young's
modulus, YM) obtained on preparation of the cross-
linked PPP matrix is dependent on the γ-irradiation
cross-linking dose as shown in Figure 1, for the low
extensions of interest in vascular prostheses. The

20   stress-strain data obtained on a band of 20 Mrad cross-
linked polypentapeptide (X^{20}-PPP) for a sequence of
cycles at 10% increments up to 90% in Figure 2 shows
the low degree of hysteresis for these initial
extensions. In this particular sample, rupture

25   occurred on approaching 100% extension, though
extensions of greater than 200% are routinely obtained
when the sample is prepared without vacuolization. The
material is significantly strengthened and is given
more body for ease of handling by compounding with a

30   polyhexapeptide, $(VAPGVG)_n$ or PHP, as in the sample
shown below in Figure 6C. A series of stress/strain
curves for the X^{20} -(PHP:PPP, 1:6) taken at larger
percent increments in Figure 3 shows the extent of
hysteresis and a rupture of this sample on approaching

35   200% extension. When the sequential multicomponent

models are prepared, i.e., $[(VPGVG)_m-(VAPGVG)_n]_\ell$, and cross-linked properties yet more favorable will be obtained than simply mixing PHP and PPP in parallel.

To more clearly illustrate the advantageous properties of the present elastomeric prostheses, comparison with natural vessels is provided. Figure 4 illustrates a series of stress-strain curves on dog femoral artery. Figure 4A is one continuous stress/strain curve up to 320% extension whereas in Figure 4B a series of cycles of 20% extension increments is shown. This series demonstrates that, for the femoral artery, there is an irreversible hysteresis for extensions above 30% presumably because collagen fibers come into tension and rupture. Figure 4C shows the stress/strain curve following a single extension to approximately 120%. In general, Figure 4 demonstrates a vessel designed for operating at small extensions and forces while having the capacity to withstand high tensile forces and extensions without rupture. In an elastomeric vascular prosthesis, the purpose of which is to provide a scaffolding for regeneration of the natural artery, what requires matching is the low extension elasticity.

These lower forces are more readily seen in Figure 5 for the elastic internal mammary artery of dog where the stress at 30% extension is 2.25 grams/mm^2 which is about 70% of the value obtained for X^{20}-PPP at 30% extension e.g., 3.2 grams/mm^2 (see Figure 2). Thus the basic synthetic elastomeric matrices have the requisite property which for X^{20}-PPP falls between the values for the internal mammary and femoral arteries. This property can be fine tuned to match well the stress/strain characteristics in the 0 to 30% extension range. It, of course, is to be appreciated that the radial dilatations and changes in length in situ

normally are less than 20% with the special exception
of the pulmonary artery of man where the radial
dilatation is 60 to 70% and the static elastic moduli
($Ep = \Delta PR/\Delta R$) vary from about 0.4×10^6 dynes/cm^2 for

5   the carotid artery of man to about 3×10^6 dynes/cm^2
for the femoral artery. Also, the active contractile
force developed by vascular muscle is in the range of 2
to 3×10^6 dynes/cm^2 for the carotid and pulmonary
arteries. These elastic properties of vascular wall

10   are attainable with the described elastomeric
polypeptide vascular materials.

        Preliminary stress/strain studies have also been
used to assess suture pull-out characteristics.
Comparison has been made with the internal mammary

15   artery of dog where single suture pull-out occurred at
a stress of 11 grams/mm^2; using the same Prolene™
suture, the value for X^{20} -(PHP:PPP, 1:6) occurred at
4.3 grams/mm^2. This was with a sample that was formed
before methods for improving uniformity of the PHP and

20   PPP mix and while vacuolization was yet a significant
factor. It is expected that suture pull-out values
similar to those of the internal mammary artery can be
obtained for the synthetic material.

        The polypentapeptide when formed as an elastomeric

25   matrix results in a very interesting biomaterial. For
vascular wall, however, a matrix of greater strength
and easier handling characteristics could be desired.
This has been achieved by combining polypentapeptide
(PPP) with polyhexapeptide (PHP) to obtain a matrix

30   such as that characterized in Figure 3. The more
gelatinous-like X^{20}-PPP (20 Mrad cross-linked
polypentapeptide matrix) on addition of PHP becomes
more teflon-like. The approach in making a section of
synthetic elastomeric tube is to dissolve PPP and PHP

35   in water at low temperature in a suitable tube (see

Figure 6A). On raising the temperature, a coacervate
phase is formed and the excess water is removed. The
pestle shown in Figure 6B is inserted into the
viscoelastic phase which flows into and fills the
5   channel in the pestle. On γ-irradiation cross-linking,
the pestle is removed from the tube and the cross-
linked matrix is removed from the pestle to obtain the
elastomeric tube in Figure 6C.

        As shown in Figure 7, the stress/strain curves of
10   the composite PHP/PPP matrix are temperature dependent;
in Figure 8A is the temperature dependence of length,
and in Figure 8B on the right-hand ordinate is the
elastic modulus (YM) as a function of temperature. The
elastic modulus is seen to increase with temperature
15   and for X^{20}-(PHP:PPP, 1:6) the elastic modulus is in
the range of 2 to 4 x 10^6 dynes/cm^2 near physiological
temperatures. Above 55°C, the matrix begins to
denature as reflected by the loss of elastic modulus.
Accordingly, a small caliber vessel (e.g., the 4.8 mm
20   ID tube shown) can be formed for consideration as a
synthetic elastomeric polypeptide vascular material and
it contains within it polypeptide that has been found
to be chemotactic toward vascular wall cells. This is
yet a primitive structure when realizing the means
25   whereby it can be fine tuned for its proposed function
as for example in the layering described below.

C. VASCULAR WALL LAYERING

        As noted above, the properties of the sequential
30   polypeptides of elastin may be varied considerably and
may be used in linear or cross-linked combination in a
single layer of material or various layers, each having
a different composition, which may be combined
advantageously.

35

The present invention will now be further illustrated by the following example which is provided for purposes of illustration only and is not intended to limit the present invention.

5

Example

Formation of an Intimal Layer: The polypentapeptide in the precross-linked state at 37°C is a sticky material which adheres to all but

10 hydrophobic surfaces. The 37°C state is 38% peptide and 62% water by weight, but on drying, it hardens and becomes glass-like. Accordingly, it is a simple process to apply a thin layer of polypentapeptide on a rotating pestle; the PPP or any variant thereof as

15 described previously, would contain, within its primary structure, cell attachment sequences for endothelial cells and as well could contain dissolved within the viscoelastic mass an appropriate concentration of chemotactic peptides for endothelial cells.

20 Additionally, as noted previously, the composition of the polypentapeptide can be varied to compensate for the effect of adding cell attachment sequences and chemotactic peptides and the temperature of the inverse temperature. While the pestle rotates, the sample

25 dries forming a layer of dried glue of desired thickness on the pestle.

Addition of a Medial Layer: The pestle with the dried intimal layer can be inserted into the tube of desired diameter which, in its bottom, contains the

30 viscoelastic mass that is to be the medial layer. This would be a PHP/PPP or $[(VPGVG)_m-(VAPGVG)_n]_\ell$ coacervate at its 37°C water composition. Once the pestle is inserted, the PHP/PPP coacervate fills the space between the intimal layer and the tube wall. The

35 intimal layer slowly hydrates but does not lose its

identity due to the extremely slow diffusion of the
high molecular weight polypeptides at compositions of
40% peptide by weight. When an additional layer is
desired,then a lower cross-linking dose, say 10 Mrad,
would result in an insoluble matrix that could be
further layered.

Generally, in the above formula, m has a value of
about 5 to 20, preferably 10 to 15, and n has a value
of 2 to 10, preferably 3 to 6. ℓ is defined
previously. However, ℓ can have a high value
corresponding to a value of (m + n) of as high as 5,000

In addition to being the primary structural
component of the vascular wall the medial layer is to
be specialized for smooth muscle cells by inclusion of
the appropriate cell attachment and chemotactic peptide
sequences.

As noted above, a dose of γ-irradiation is used
which is, generally, the lowest possible dose required
to match the compliance of the vascular wall. However,
doses in excess of 30-40 Mrad are not generally used.

Addition of an Adventitial Layer: Using a larger
tube, the cylinder with intimal and medial layers
surrounding the pestle at 37°C is inserted into a
larger tube in which a lysine containing PPP coacervate
has been placed. This material then flows around the
medial layer to form a third layer. On exposing this
tube to another 10 Mrad, the inner layers will have
received the desired 20 Mrad and an outer, looser layer
will have been added. As the lysine-containing
polypentapeptide or polytetrapeptide is a substrate for
the extracellular enzyme, lysyl oxidase, the potential
exists for the adventitial layer to become covalently
cross-linked to the extracellular matrix surrounding
the implant site. This is because oxidation of lysine
by lysyl oxidase to form the aldehyde is the natural

mechanism for cross-linking in the extracellular
matrix.

   As noted, any additional method of chemical cross-
linking using cross-linking units capable of being
5 cross-linked by lysyl oxidase may be used as described
in U.S. patent 4,589,882.

   A schematic drawing of the tube resulting from the
layering discussed above is shown in Figure 9 where the
discussed compositions are also indicated. For the
10 intimal and adventitial layers, the sequences as
pentameters (VPGVG), tetramers (VPGG) or hexamers
(VAPGVG) are added in the desired ratio and then
polymerization is carried out such that the
incorporation along the sequence is random to obtain a
15 polymer of greater than 50 kD. For the medial layer,
the sequences may be incorporated as desired in a
single long sequence of the order of 100 residues, and
then this unit is polymerized to a greater molecular
weight. As an example of a medial layer, can be
20 mentioned a layer which is obtained by appropriately
mixing polyhexapaptide and polypentapeptide, each of
greater than 50 kD, which are cross-linked
by γ-irradiation.

   This tube is shown in Figure 6C. It is this
25 material that was characterized in Figure 3 (above) and
was further characterized in Figures 7 and 8; it is
designated X^{20}-(PHP:PPP, 1:6) as the ratio of
polyhexepeptide to polypentapeptide used for cross-
linking was 1:6 by weight.

30 Further, at the junction between the intimal and
medial layers or layered within the medial matrix, can
be included a porous layer to facilitate migration of
smooth muscle cells. This can be achieved by addition
of a fine coral or sized particulate carbonate powder
35 to a semi-dry, tacky surface of the coated pestle at

any desired radius as long as the γ-irradiation dose is
10 Mrad or less. On drying, the treated pestle with
its layers would be introduced into a tube of desired
diameter with the PHP/PPP viscoelastic coacervate of

5   the proper volumn in the bottom. On completing the 20
to 25 Mrad dose, the synthetic tube could be removed
and treated with acid of sufficient strength to
dissolve the carbonates but not of a strength to
hydrolyze the peptide.

10   Additionally, it is noted that instead of using
$\{VPGVG\}$ in the basic sequential polypeptide, it is also
possible to use generally a pentapeptide of the formula
$\{R_1PR_2R_3G\}$ as already defined previously. Similarly,
instead of using $\{VAPGVG\}$ in the basic sequential

15   polypeptide, the variations described previously may
also be utilized.

D.   STIMULATION OF CELLS ON THE SURFACES OF
      ELASTOMERIC MATRICES

20   In accordance with yet another important aspect of
the present invention, the elastomeric materials
described herein may be advantageously used as vascular
prostheses for the following additional reason. In
particular, when cells, such as smooth muscle cells
and/or endothelial cells, are placed on the surface of

25   the present elastomeric vascular prosthesis, and
subjected to stretch/relaxation cycles, it is found
that the cells are stimulated to produce the
macromolecules which make up the vascular wall. In
other words, the cells are stimulated to produce their

30   own vascular wall.

The above aspect of the present invention is, in
part, based upon the earlier work of Glagov who
demonstrated that elastic substrates might be used to
stimulate cell synthesis of matrix components. See

35

Exp. Cell Research, 109, 285-298 (1977); Science, 191, 475-477 (1976); and Circ. Res., 14, 400-413 (1964) by Glagov et al. However, none of this work was conducted with the present elastomeric materials.

Further, the biodegradability and biocompatibility of the present vascular prostheses also appear to be advantageous in that it has been demonstrated that compliance and biodegradation of vascular grafts stimulates the regeneration of elastic laminae in neoarterial tissue. See Cell Tissue Res., 242, 569-578 (1985), by van der Lei et al. Thus, the present vascular prostheses have the advantageous effect of stimulating the synthesis of cell matrix components, such as collagen and new elastic fibers, to thereby regenerate vascular walls.

Having now fully described this invention, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

Claims

1. A vascular prosthetic material having at least
one layer containing an elastomeric material capable of
reversibly contracting and relaxing by an inverse
temperature transition.

2. The vascular prosthetic material of Claim 1,
which is in the form of a vessel suitable for use as a
portion of an artery, vein or lymphatic vessel.

3. The vascular prosthetic material of Claim 1,
which is substantially cross-linked.

4. The vascular prosthetic material of Claim 1,
which comprises an elastomer containing repeating units
selected from the group consisting of elastomeric
tetrapeptide and pentapeptide repeating units and units
thereof modified by hexapeptide units and mixtures
thereof, wherein said repeating units comprise amino
acid residues selected from the group consisting of
hydrophobic amino acid and glycine residues, and
wherein said repeating units exist in a conformation
having a β-turn, which comprises a polypentapeptide
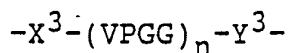unit of the formula:

$$-X^1-(VPGVG)_n-Y^1-$$

wherein

V is a peptide-forming residue of L-valine;

P is a peptide-forming residue of L-proline;

G is a peptide-forming residue of glycine; and
wherein X^1 is PGVG, GVG, VG or G or a covalent bond;
Y^1 is VPGV, VPG, VP or V or a covalent bond; and n is
an integer from 1 to about 5,000, or n is 0, with the
proviso that X^1 and Y^1 together constitute at least one
of said pentameric unit.

5. The vascular prosthetic material of Claim 1,
which comprises an elastomer containing repeating units
selected from the group consisting of elastomeric
tetrapeptide and pentapeptide repeating units and units

thereof modified by hexapeptide repeating units and
mixtures thereof, wherein said repeating units comprise
amino acid residues selected from the group consisting
of hydrophobic amino acid and glycine residues, and
wherein said repeating units exist in a conformation
having a β-turn which comprises a polypentapeptide unit
of the formula:

$$-X^1-(IPGVG)_n-Y^1-$$

wherein

I is a peptide-forming residue of L-isoleucine;

P is a peptide-forming residue of L-proline;

G is a peptide-forming residue of glycine;

V is a peptide-forming residue of L-valine; and
wherein X^1 is PGVG, GVG, VG, G or a covalent bond;
Y^1 is IPGV, IPG, IP, I or a covalent bond; and n is an
integer from 1 to 5,000, or n is 0, with the proviso
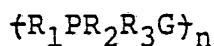that X^1 and Y^1 together constitute at least one of said
pentameric unit.

6.   The vascular prosthetic material of Claim 1,
which comprises an elastomer containing repeating units
selected from the group consisting of elastomeric
tetrapeptide and pentapeptide repeating units and units
thereof modified by hexapeptide repeating units and
mixtures thereof, wherein said repeating units comprise
amino acid residues selected from the group consisting
of hydrophobic amino acid and glycine residues, and
wherein said repeating units exist in a conformation
having a β-turn, which comprises:

A)   a polypentapeptide unit of the formula:

$$-X^1-(IPGVG)_n-Y^1-$$

and

B)   a polypentapeptide unit of the formula:

$$-X^2-(VPGVG)_n-Y^2-$$

wherein for the above formula,

I is a peptide-forming residue of L-isoleucine;

P is a peptide-forming residue of L-proline;

G is a peptide-forming residue of glycine;

V is a peptide-forming residue of L-valine; and
wherein X^1 and X^2 are each PGVG, GVG, VG, G or a
covalent bond; Y^1 is IPGV, IPG, IP, I or a covalent
bond; and n in both formulas is an integer from 1 to
5,000, or n is 0, with the proviso that X^1 and Y^1 or X^2
and Y^2 together constitute at least one of said
pentameric unit.

7. The vascular prosthetic material of Claim 1,
which comprises an elastomer containing repeating units
selected from the group consisting of elastomeric
tetrapeptide and pentapeptide repeating units and units
thereof modified by hexapeptide repeating units and
mixtures thereof, wherein said repeating units comprise
amino acid residues selected from the group consisting
of hydrophobic amino acid and glycine residues, and
wherein said repeating units exist in a conformation
having a ß-turn which comprises a tetrapeptide of the
formula:

$$-X^3-(VPGG)_n-Y^3-$$

wherein

X^3 is PGG, GG, G or a covalent bond;
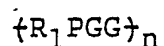
Y^3 is VPG, VP, V or a covalent bond;
and V is a peptide-producing residue of L-valine; P is
a peptide-producing residue of L-proline; and G is a
peptide-producing residue of glycine; and n is an
integer from 1 to 5,000, or n is 0, with the proviso

that X^3 and Y^3 together constitute at least one of said tetrameric unit.

8. The vascular prosthetic material of Claim 1, which comprises an elastomer containing repeating units selected from the group consisting of elastomeric tetrapeptide and pentapeptide repeating units and units thereof modified by hexapeptide repeating units and mixtures thereof, wherein the repeating units comprise hydrophobic amino acid residues and glycine residues, and wherein the repeating units exist in a conformation having a β-turn which comprises a tetrapeptide of the formula:

$$-X^4-(IPGG)_n-Y^4-$$

wherein X^4 is PGG, GG, G or a covalent bond; Y^4 is IPG, IP, I or a covalent bond; and

I is a peptide-producing residue of L-isoleucine;

P is a peptide-producing residue of L-proline; and

G is a peptide-producing residue of glycine; and n is an integer from 1 to 5,000, or n is 0, with the proviso that X^4 and Y^4 together constitute at least one of said repeating tetrameric unit.

9. The vascular prosthetic material of Claim 1, which comprises an elastomer containing repeating units selected from the group consisting of elastomeric tetrapeptide and pentapeptide repeating units and units thereof modified by hexapeptide repeating units and mixtures thereof, wherein the repeating units comprise hydrophobic amino acid and glycine residues, and wherein the repeating units exist in a conformation having a β-turn which comprises a pentapeptide repeating unit of the formula:

$$\text{+}R_1PR_2R_3G\text{+}_n$$

wherein R_1 is a peptide-producing residue selected from
the group consisting of Phe, Leu, Ile, and Val; R_2 is
such a residue selected from the group consisting of
Ala, D-Ala and Gly; R_3 is such a residue selected from
the group consisting of Phe, Leu, Ile, and Val; P is a
L-proline-producing residue, and G is a glycine-
producing residue, and n is an integer from 1 to about
5,000.

10. The vascular material of Claim 1, which
comprises an elastomer containing repeating units
selected from the group consisting of elastomeric
tetrapeptide and pentapeptide repeating units and units
thereof modified by hexapeptide repeating units and
mixtures thereof, wherein said repeating units comprise
amino acid residues selected from the group consisting
of hydrophobic amino acid and glycine residues and
wherein said repeating units exist in a conformation
having a ß-turn, which comprises a tetrapeptide
repeating unit of the formula:

$$\text{+R}_1\text{PGG+}_n$$

wherein R_1 is a peptide-producing residue selected from
the group consisting of Phe, Leu, Ile and Val; P is a
L-proline-producing residue, and G is a glycine-
producing residue; and n is an integer from 1 to about
5,000.

11. The vascular material of Claim 1, which
comprises intermixed with elastomeric repeating
sequences, at least one hexapeptide repeating unit of
the formula:

$$\text{+APGVGV+}$$

wherein

A is a peptide-forming residue of L-alanine; or in place thereof a peptide-forming residue selected from the group consisting of Val, Ile, Phe and Leu;

P is a peptide-forming residue of proline; or in place thereof a peptide-forming residue selected from the group consisting of Val, Ile, Phe, Leu and Ala;

G is a peptide-forming residue of glycine; or in place thereof of alanine;

V is a peptide-forming residue of valine; or in place thereof a peptide-forming residue selected from the group consisting of Phe, Leu, Ile and Ala;

12. The vascular material of Claim 1, which comprises three layers, each of which contain said elastomeric material.

13. The vascular material of Claim 12, which is in the form of a tube or vessel, and wherein said three layers comprise:

a) an intimal-inner layer, which comprises an elastomeric material and one or more cell attachment sequences for endothelial cells, and one or more chemotactic peptides for endothelial cells or a combination thereof;

b) a medial-middle layer, which comprises an elastomeric material and one or more cell attachment sequences for smooth muscle cells, and one or more chemotactic peptides for smooth muscle cells; and

c) an adventitial-outer layer, which comprises an elastomeric material and one or more lysine-containing polypentapeptides which render said outer layer capable of being covalently cross-linked to extracellular matrix surrounding said vascular material when implanted.
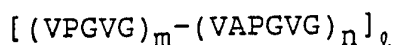
14. The vascular material of Claim 13, wherein the elastomeric material of said intimal-inner layer comprises the elastomer of Claim 4.

15.   The vascular material of Claim 13, wherein
the elastomeric material of said intimal—inner layer
comprises the elastomer of Claim 5.

16.   The vascular material of Claim 13, wherein
the elastomeric material of said intimal—inner layer
comprises the elastomer of Claim 6.

17.   The vascular material of Claim 13, wherein
the elastomeric material of said intimal—inner layer
comprises the elastomer of Claim 7.

18.   The vascular material of Claim 13, wherein
the elastomeric material of said intimal—inner layer
comprises the elastomer of Claim 8.

19.   The vascular material of Claim 13, wherein
the elastomeric material of said intimal—inner layer
comprises the elastomer of Claim 9.

20.   The vascular material of Claim 13, wherein
the elastomeric material of said intimal—inner layer
comprises the elastomer of Claim 10.

21.   The vascular material of Claim 13, wherein
the elastomeric material of said medial-middle layer
comprises an elastomer having the formula:

$$[(VPGVG)_m-(VAPGVG)_n]_\ell$$
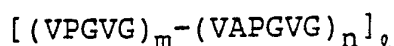
wherein m is in the range of about 5 to 20, and n is in
the range of about 2 to 10, and ℓ has a value such that
a molecular weight of at least about 10,000 daltons is
obtained.

22.   The vascular material of Claim 21, wherein
said elastomer has the formula:

$$[(VPGVG)_m-(VAPGVG)_n]_\ell$$

wherein m is in the range of 10 to 15, and n is in the
range of 3 to 6, and ℓ has a value such that a

molecular weight is obtained in the range of about
10,000 to 50,000 daltons.

　　　23.　The vascular material of Claim 13, wherein
the elastomeric material of said adventitial-outer
layer comprises an elastomer having the formula:

$$[(VPGVG)_n-(VPGKG)]_\ell$$
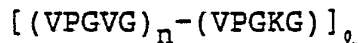
wherein K is a lysine-containing peptide-forming
residue, n has a value of about 5 to 30, and ℓ has a
value such that a molecular weight of at least 10,000
daltons is obtained.

　　　24.　The vascular material of Claim 23, wherein
the elastomer has the formula:

$$[(VPGVG)_n-(VPGKG)]_\ell$$
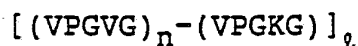
wherein n has a value to about 10 to 20, and ℓ has a
value such that a mulecular weight of at least about
10,000 to 50,000 daltons is obtained.

　　　25.　The vascular material of Claim 13, which
further comprises between said intimal and said medial
layers a porous layer comprising an elastomeric
material containing an amount of a fine coral or sized
particulate carbonate powder thereto to facilitate
migration of smooth muscle cells.

YOUNG'S MODULUS AT LOW EXTENSIONS
γ-IRRADIATED POLYPENTAPEPTIDE

FIG. 1

X^{20}-POLYPENTAPEPTIDE
STRESS=3.2 GRAMS/mm^2
AT 30% EXTENSION

FIG. 2

χ^{20}-(PHP·PPP, 1:6)
STRESS=2.3g/mm^2
AT 30% EXTENSION

FORCE, GRAMS

18

12

6

0

0   60   120   180
% ELONGATION

FIG. 3

40   DOG INTERNAL MAMMARY ARTERY

STRESS=2.3g/mm^2
AT 30% EXTENSION

FORCE, GRAMS

20

0

0   40   80
% ELONGATION

FIG. 5

DOG FEMORAL ARTERY (PREVIOUSLY UNSTRESSED SAMPLE)

FIG. 4A

DOG FEMORAL ARTERY (PREVIOUSLY UNSTRESSED SAMPLE)

STRESS = 4.7g/mm² AT 30% EXTENSION

FIG. 4B

DOG FEMORAL ARTERY (PREVIOUSLY STRESSED SAMPLE)

231.75 GRAMS/mm²

FIG. 4C

4/6

FIG. 6A   FIG. 6B   FIG. 6C

AT EACH TEMPERATURE ELONGATION IS TO 20%
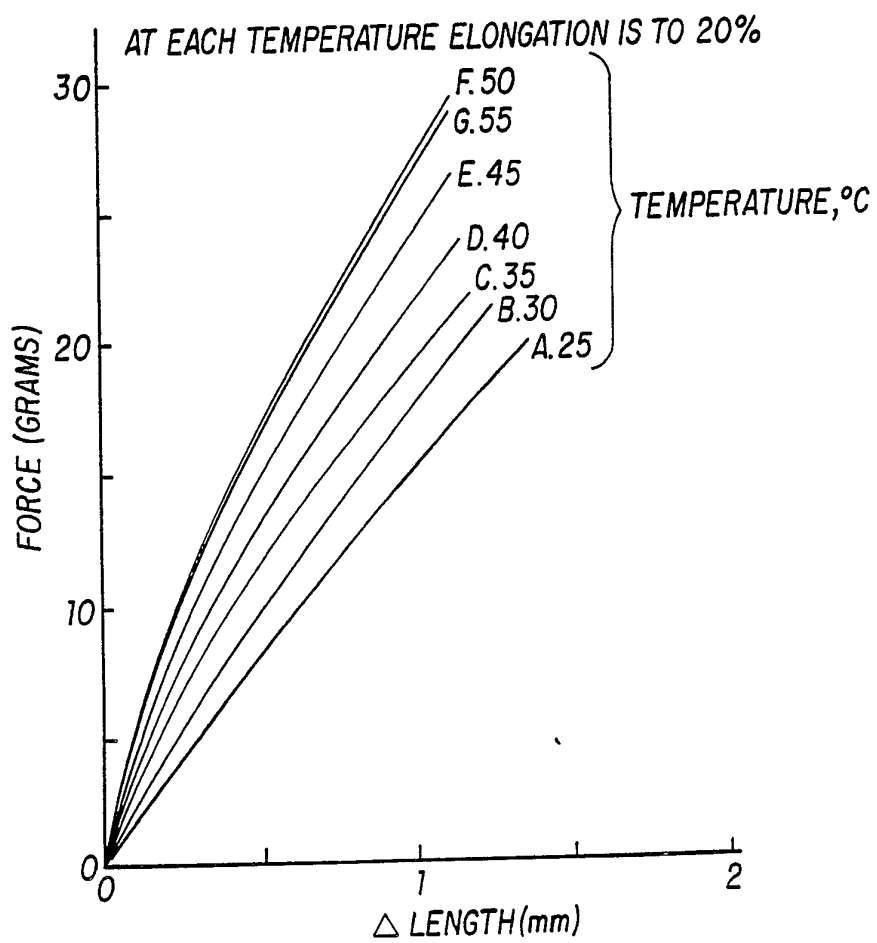
FIG. 7

FIG. 8A
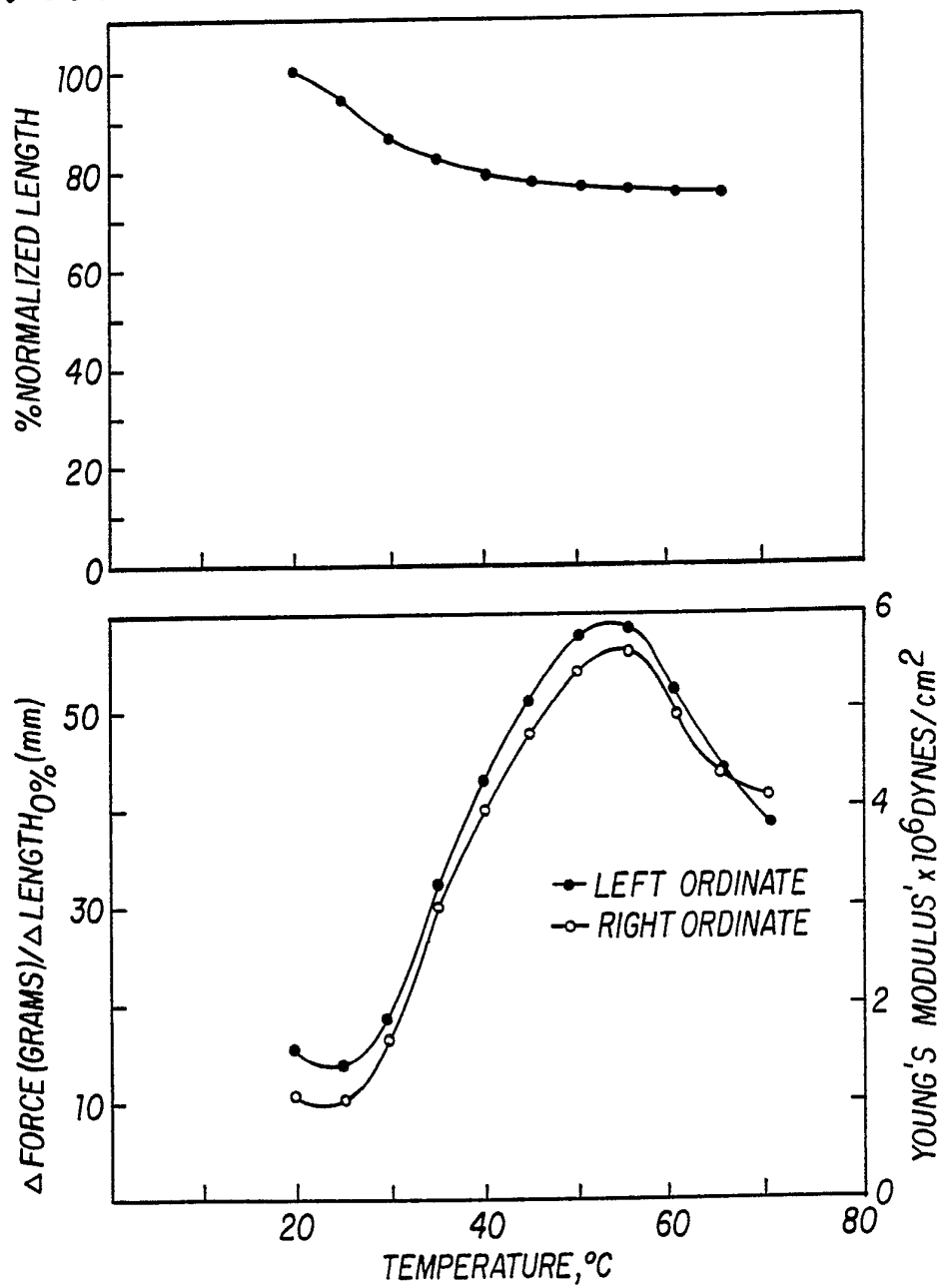
FIG. 8B

FIG. 9

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

PCT(4) A 61F2/02; A61 F 2/06; A61K 37/02; CO 7K 5/02
  US   514/17,18; 530/323,324,324,330,350; 623/1,11,66

II. FIELDS SEARCHED

	Minimum Documentation Searched [7]
Classification System	Classification Symbols
U.S	514/17,18 623/1,11,66 530/323,324,328,330,350

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US,A 4,132,746 (Urry et al.) 02 January 1979 See Entire Document	1-25
X	US,A 4,474,851 (Urry) 02 October 1984 See Entire Document	1-25
X	US,A 4,500,700 (Urry) 19 February 1985 See Entire Document	1-25
X	US,A 4,589,882 (Urry) 20 May 1986 See Entire Document	1-25
X,P	US,A 4,783,523 (Urry) 08 November 1988 See Entire Document	1-25

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 June 1989	25 AUG 1989
International Searching Authority	Signature of Authorized Officer
ISA/US	Lester L. Lee

Form PCT/ISA/210 (second sheet) (Rev.11-87)