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(54) Title: SYNTHETIC COMPOUNDS AND COMPOSITIONS WITH ENHANCED CELL BINDING

(57) Abstract

Compositions of the invention include composites comprising a biomaterial carrying compounds with enhanced cell binding with respect to collagen. These composites are useful for soft and hard tissue repair or reconstruction. Suitable compounds with enhanced cell binding include synthetic peptides that mimic the conformation necessary for recognition and docketing of collagen binding species (such as cell surface receptors for collagen and fibronectin). Hydrogel matrices as the biomaterial promote cell attachment to the matrix and cell migration into the matrix.

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SYNTHETIC COMPOUNDS AND COMPOSITIONS WITH ENHANCED CELL BINDING

Field of the Invention

The present invention generally relates to compounds that mimic a small biologically active segment of collagen, and more particularly relates to compounds such as synthetic peptides that have enhanced cell binding with respect to collagen and that are usefully combined with matrices to form composites for growing vertebrate cells.

Background of the Invention

Collagen is the most abundant protein found in vertebrates. Approximately 25 percent of all animal protein is collagen. Collagen is unusual among proteins in that the amino acid glycine constitutes one-third of the total amino acid content and occurs at nearly every third amino acid residue. Also, many proline amino acids are found in collagen. Collagen contains two amino acids present in very few other proteins, i.e., hydroxyproline and hydroxylysine. The sequence glycine-proline-hydroxyproline recurs frequently.

Because glycine is a very small amino acid, chains of collagen can wind tightly around one another to form a triple helix. The side chains of proline form cross-links that lock the three strands together. Additionally, mature collagen frequently contains carbohydrate units covalently attached to its hydroxylysine residues. A disaccharide of glucose and galactose is commonly found attached to strands of collagen. Still other forms of collagen can form planar sheets, which are rich in carbohydrates.

Collagen functions as a structural protein of tissues. It is the major fibrous element in skin, cartilage, bone, tendon, teeth, and blood vessels. Collagen is present to some extent in nearly all organs and serves to hold cells together in discrete units. It forms insoluble fibers that have a high tensile strength. Furthermore, the basic structure of collagen is modified to meet the specialized needs of particular tissues, and these are reflected in the various types of collagen that have been identified.

The several types of collagen are a family of genetically related proteins that exhibit fundamentally similar secondary and tertiary protein structures. As used herein unless otherwise specified, "collagen" refers to any of the known types of collagens.

Type I collagen, the most prevalent type and the species found in skin, tendon, bone, and cornea, is comprised of two chains of one kind, termed $\alpha 1(I)$, and one of another, termed $\alpha 2(I)$. Other types of collagen have three identical chains. Each of the three strands consists of about 1,000 amino acid residues, and each has a helical conformation. The three strands wind around each other to form a superhelical cable and are hydrogen bonded to each other. As mentioned above, this structure is possible because of the presence and regularity of glycine units.

Studies have shown that the presence and concentration of imino residues, proline and hydroxyproline, are essential for generating and stabilizing the triple helical conformation of collagen.

In short, the stability of the helical form of a single strand of collagen depends on the locking effect of proline and hydroxy-proline residues. The triple helix is further stabilized by transverse hydrogen bonding and van der Waals interactions between residues on different strands. The superhelix is

sterically allowed because glycine occupies every third position in the amino acid sequence.

In addition to being a major determinant of the architecture and tensile strength of tissues, collagen participates in numerous physiologically important interactions. These include, but are not limited to, the formation of complexes with other macromolecules such as fibronectin, the modulation of cell proliferation, the mediation of cell migration and differentiation, and the modulation of specific gene expression.

In order for such interactions to occur, the molecules on the surface of collagen fibers must exhibit molecular perspectives that are specific for recognition. This requires local conformational changes. It has been suggested that the binding of certain cells, such as platelets, may involve a conformationally perturbed region of the α1 chain of collagen, which is located approximately one-quarter of the length of the chain from the C-terminus.

Previous studies have shown that the three amino acid sequence, Arg-Gly-Asp, found in a variety of proteins, including collagen, may play a major role in the binding of cells. This sequence appears twice within the α1(I) chain, and one of those occurrences is within the conformationally perturbed region described above.

Collagen fragments and synthetic peptide sequences corresponding to portions of collagen have been prepared and studied. Nagai et al. prepared eleven synthetic peptides by solution procedures to study substrate specificity of purified tadpole collagenase, with the synthesized peptides having the same or closely similar sequences to that occurring around the Gly-Ile bond in the position 772-773 of the α_1 chain. The authors proposed an eight amino acid peptide (with

acetyl at the N-terminus and esterified at the C-terminus) as the best substrate for vertebrate collagenase. Nagai et al., Biochimica et Biophysica Acta, 445, 521-524 (1976).

Collagen has been suggested in mixtures or combinations with bone minerals, such as is discussed in U.S. Patent No. 4,992,226, inventors Piez et al., issued February 12, 1991. Collagen has also been suggested in combination with hydrogels for cornea implants, as illustrated by U.S. Patent No. 4,994,081, inventors Civerchia et al, issued February 19, 1991. Skin and nerve tissue repairs have been suggested through use of endodermal implants and artificial epidermis fashioned out of collagen and mucopolysaccharide, as illustrated by U.S. Patent No. 4,060,081, inventors Yannas et al., issued November 29, 1977.

However, the present materials and composites presently employed or suggested as tissue implants or for tissue repair have various shortcomings. Collagen itself appears to cause some adverse reactions within the body. Also, the manner in which collagen is reconstituted during preparations of the combinations with bone minerals, hydrogels, and so forth, tends to markedly alter the normal collagen biological activity and apparently masks some of the biologically active sites.

Summary of the Invention

A family of synthetic peptides that mimic the cell binding domain of collagen can attach cells to various substrates, or matrices. The cell binding domain of these compounds has enhanced cell binding with respect to collagen. The domain includes a core sequence that, at physiologic conditions, is folded in a ß-bend with the ß-bend being formed at -Ile-Ala-. An embodiment of the family includes six amino acid

residues with the sequence Gln-Gly-Ile-Ala-Gly-Gln (SEQ ID NO:3). The peptides may be carried by a matrix as a composite useful in growing cells. Such composites are preferably formed from biomaterials and have properties of promoting cell attachment to the matrix and promoting cell migration into the matrix when the matrix is porous. Among applications for composites of the invention are bone repair and tooth implants in reconstructive surgeries.

Brief Description of the Drawings

Figure 1 is the photograph of an agarose gel on which fibroblast cells were placed and after 24 hours died. The agarose gel on which the cells were placed was a prior art gel.

Figure 2 is the photograph of an inventive embodiment in which an agarose gel included an inventive peptide. Fibroblast cells were placed on the gel surface, and commenced to grow and to migrate into the gel itself.

Detailed Description of the Invention and Preferred Embodiments

Novel synthetic compositions of matter are described in the present invention. These include compounds that are structurally or biologically analogous to a small region of collagen and mimic the conformation recognized by collagen binding species. The region from which synthetic peptides of the invention have been designed is sometimes referred to as "P-15", includes all or part of 15 amino acid residues, Gly-Thr-Pro-Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg-Gly-Val-Val, of the α1(I) chain of collagen, and spans approximately residues 766-780 of this chain. The P-15 region does not occur as a natural fragment of collagen nor is it a product of natural enzymatic cleavages.

Collagen exists in a very tightly coiled superhelical structure, wherein its tensile strength is stabilized by the high content of imino residues. Thus, for interactions with other cells or compounds, the collagen structure must be perturbed. Thermal motion can overcome the forces that stabilize the triple-stranded helix, yielding a disrupted structure. An advantage of the present invention is that, given the size and structure of the synthetic compositions disclosed herein, there is no need for such conformational perturbations. Compounds of the invention not only can mimic the biological activity of collagen, but also can exhibit cell binding functions enhanced with respect to collagen. The amino acid sequences of synthetic peptides in accordance with the invention need not correspond precisely to the P-15 region, but rather may include (indeed, sometimes preferably only include) a portion of it.

The P-15 region represents half of one turn of the collagen triple helix, i.e. fifteen residues, which is believed to be exposed in intact collagen molecules on the surface of fibers. The other half of the turn faces the core of the fiber. Theoretical and experimental studies showed that the sequence contained in P-15 can acquire a conformation dramatically different from the triple helical conformation generally observed in the rest of the collagen molecule. This atypical, or "non-collagen", conformation is believed necessary for recognition by and the docking of collagen binding species, such as cell surface receptors for collagen and fibronectin. The three dimensional surface presented by the P-15 region or parts of the P-15 region is complementary to the reactive surface present on the binding species (receptors, fibronectin). Compounds of the invention mimic this surface of collagen, and any

compounds that can generate a similar surface can be expected to have similar biological activity.

An embodiment of the present invention involves synthetic compositions that have a biological activity functionally comparable to that of all or some portion of P-15. By "functionally comparable," is meant that the shape, size, and flexibility of a compound is such that the biological activity of the compound is similar to the P-15 region, or a portion thereof. Biological activities that may be possessed by the peptide include inhibition of collagen synthesis, inhibition of collagen binding, and inhibition of cell migration. Of particular interest to the present invention is the property of enhanced cell binding. Useful compounds should be selected on the basis of similar spacial and electronic properties as compared to P-15 or a portion thereof. These compounds typically will be small molecules of 100 or fewer amino acids or in the molecular weight range of up to about 5,000 daltons, more typically up to 2,500 daltons. Inventive compounds will be illustrated with synthetic peptides; however, nonpeptides mimicking the necessary conformation for recognition and docking of collagen binding species are also contemplated as within the scope of this invention. For example, cyclic peptides on other compounds in which the necessary conformation is stabilized by nonpeptides (e.g., thioesters) is one means of accomplishing the invention.

It is the central portion, forming a core sequence, of the P-15 region that is essential for the desired collagen-like activity. Thus, peptides of this invention preferably contain the sequence Gly-Ile-Ala-Gly (SEQ ID NO:9). The two glycine residues flanking the fold, or hinge, formed by -Ile-Ala- are hydrogen bonded at physiologic conditions and thus stabilize the ß-fold. Because the stabilizing hydrogen bond between

glycines is easily hydrolyzed, two additional residues flanking this sequence can markedly improve the cell binding activity by further stabilizing the bend conformation. For example, addition of glutamine at each end, as represented by Gln-Gly-Ile-Ala-Gly-Gln (SEQ ID NO:3) markedly improves the activity and is a synthetic peptide of this invention. In fact, this six amino acid (SEQ ID NO:3), is as active, or more active, than a peptide having the entire P-15 region. A nonapeptide Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Thr (SEQ ID NO:2) is also equally as active as the entire P-15 region. On the other hand, if there is cleavage in the middle of the Gly-Ile-Ala-Gly active site, activity is lost. These results (and other tests) clearly demonstrate the importance of the central 4 to 6 residues. The core sequence accordingly should have five or six amino acid residues, preferably six or more.

A number of synthetic peptides have been prepared and are shown in Table 1.

TABLE 1

Peptides	SEQ ID NO:
Gly-Thr-Pro-Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg-Gly-Val-Val	1
Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg	2
Gln-Gly-Ile-Ala-Gly-Gln	3
Gln-Gly-Ile-Ala-Gly-Gln-Arg	4
Phe-Gly-Ile-Ala-Gly-Phe	5
Gly-Ile-Ala-Gly-Gln	6
Gln-Gly-Ala-Ile-Ala-Gln	7

Although SEQ ID NO:6 has only five amino acid residues, it has a cell-binding activity (relative to the SEQ ID NO:1 peptide) of 70%. However, another five amino acid peptide prepared (Gln-Gly-Ile-Ala-Gly (SEQ ID NO:8)) only had a cell-binding activity relative to the SEQ ID NO:1 peptide of 42%, which means it is slightly enhanced with respect to collagen, but not significantly enhanced.

The synthetic peptide identified in Table 1 as "SEQ ID NO:7" differs from the other family members in having the essential -Ile-Ala- hinge flanked by alanine and glutamine, respectively. This is believed due to the formation of a hydrogen bond between the alanine and glutamine that stabilizes the essential ß-fold.

In theoretical studies those peptides having the SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3 showed a high potential for a conformation in which the peptide is folded in a ß-bend at the -Ile-Ala- portion. Solution conformations in a variety of membrane-mimetic environments showed that a folded sheet-like structure is generated by these peptides. As the side chains of Gln retain hydrophobic character, a stabilizing interaction ensues and the bend structure is stabilized. This concept was confirmed by synthesizing the

hexapeptide having the SEQ ID NO:5 as an analogue, which exhibited cell binding activity comparable to that of the peptides of SEQ ID NOS:1, 2, 3, and 5. Thus, the molecular shape and stabilizing interactions are critical for the desired activity. The importance of the residues -Ile-Ala- in the middle of the ß-bend of the core sequence is emphasized by the lack of activity in a peptide analogous to the peptide of SEQ ID NO:7, but with Ala of the -Ile-Ala- bend replaced by Gly.

To briefly summarize, synthetic peptides of this invention must have a core sequence that has -Ile-Ala- formed in a ß-bend at physiological conditions. This ß-bend of the core sequence means that the domain of the inventive synthetic peptides has an ability to exist in that conformation, either due to allosteric induction, or interaction, or more preferably by sufficient flanking amino acid residues to substantially stably hold the bend, such as by flanking hydrogen bonded residues. This core sequence must consist of four, and preferably at least five or six, amino acid residues for synthetic peptides. Additional flanking amino acid residues, up to a total of about 15, if present will typically together comprise a domain that is inhibitory of collagen bonding to cells and that has enhanced cell binding with respect to collagen. The flanking moieties facilitate the "presentation" of the ß-fold conformation in receptor situations. Because of the enhanced cell binding property for synthetic peptides of this invention, cell attachment is promoted. Additional amino acid residues or other moieties may be added to one or the other side of this domain to facilitate coupling or the like, so long as the essential cell-binding property of the domain is not substantially inhibited.

Synthetic compounds of this invention also have one or more of the following properties: they

promote cell migration into porous lattices; they bind to collagen receptors; they induce metalloproteinases; they can down-regulate prolyl hydroxylase and collagen; they inhibit cell binding to collagen; and they inhibit cell migration in vitro. The enumerated properties (including promotion of cell attachment) of synthetic peptides for the inventive family can be utilized to convey these highly desirable properties to composites for a wide variety of uses. The down-regulation of prolyl hydroxylase is of particular interest because it represents a key step in collagen synthesis. This means that compounds of the invention can be used as inhibitors of collagen synthesis to block formation of scar tissue and thus promote scarless healing.

Synthetic peptides of the inventive family are preferably substantially free of the following: glycosylation, association with other peptide chains, cross-linking, and hydroxylation, which tend to be present in naturally occurring collagen. By "substantially free," it is meant less than the average amount found in naturally occurring collagen. Peptides of the invention are preferably also substantially free of blocking groups (often used during peptide synthesis), such as t-butyloxycarbonyl group ("BOC").

Synthetic peptides that have the desired biological activities may be produced by either of two general approaches.

Polypeptides having fewer than about 100 amino acids, usually fewer than about 50 amino acids, and more usually fewer than about 25, may be synthesized by the well-known Merrifield solid-phase chemical synthesis and modifications thereof method wherein amino acids are sequentially added to a growing chain.

However, the synthetic peptides of the present invention may be synthesized by recombinant techniques involving the expression in cultured cells of

recombinant DNA molecules encoding the gene for a desired portion for the α1(I) strand of collagen. The gene encoding the desired portion of the α1(I) strand of collagen itself may be natural or synthetic. Conveniently, polynucleotides may be synthesized by well-known techniques.

In yet another embodiment, monoclonal antibodies may be raised against an epitopic region defined by P-15 or a portion thereof, or by any of the compounds of the present invention, wherein the epitopic region is responsible for binding and biological activity. By then raising antibodies against the first antibody, the binding region of the anti-idiotypic antibodies may provide functional analogs of P-15, or a functionally similar compound.

The present invention also includes composites and methods of use for promoting vertebrate cell adhesion comprising attaching any of the above-described compositions of matter to a substrate (that is, a matrix) and adding cells to the composite. Substrates include, but are not limited to, glass, plastics, hydroxyapatite, ceramics, organic polymers, gels, and silica. Preferred types of cells to be adhered include fibroblasts; however, most, if not all, cell types may be used.

The mode of attachment can be via covalent linkages, noncovalent interactions, or nonspecific adsorption. Covalent linkages include, but are not limited to, those involving ester, amide, or ether. An exemplary method of covalent linkages involves peptides of the present invention with additions of nonnatural amino acids at either the N-terminus or C-terminus to provide for binding or conjugation of the peptide to a solid phase or another protein. For example, a cysteine sequence may be added to either terminus to facilitate coupling to a carrier. Hydrophobic residues or lipid-

containing moieties, such as amino acids containing hydrophobic side chains, may be added to enhance liposome or membrane binding. When the necessary domain includes other noninterfering moieties or spacer arms (such as to facilitate binding) then the overall size of the cell attaching compound, or peptide, will usually be increased to greater than 15 amino acids of the P-15 region.

The substrate of choice may be pretreated with CNBr or other activating reagents to facilitate coupling of the composition to the substrate. Noncovalent interactions and nonspecific adsorption typically would involve the direct application of a solution containing the present compositions to the substrate. These methods of use have many applications, e.g., culturing cells to study their physiology and to make specific compounds, etc. A few examples will be more fully described hereinafter with other uses and advantages being readily apparent to one of ordinary skill in the art.

The following examples are intended to be merely illustrative of the present invention and are not to be read as limiting. The relative efficiencies of some synthetic peptides of this invention for cell-binding activity are set out in Table 1.

TABLE 1

	SEQ ID NO:	Relative Cell-Binding Activity
Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg	2	100
Gln-Gly-Ile-Ala-Gly-Gln	3	120
Gln-Gly-Ile-Ala-Gly-Gln-Arg	4	60
Phe-Gly-Ile-Ala-Gly-Phe	5	87
Gly-Ile-Ala-Gly-Gln	6	70
Gln-Gly-Ala-Ile-Ala-Gln	7	70

EXAMPLE 1

Referring to Figs. 1 and 2, a prior art agarose gel was prepared with an agarose concentration of 2 mg/ml and between 100,000-200,000 fibroblast cells were placed on the gel surface. The agarose gel had been prepared with a suitable fibroblast cell growth medium and the cells placed on the agarose gel were treated with growth medium; however, the cells died within 48 hours, as is illustrated by the Fig. 1 photograph. By contrast, when 100 μg of the SEQ ID NO:1 peptide was included in an otherwise analogous preparation of agarose gel and fibroblast cells were again placed on top, then the cells not only grew, but actually migrated into the gel itself, as is illustrated by the Fig. 2 photograph taken 72 hours after plating. This was quite surprising because cells normally do not migrate well into gels. The fibroblasts that grew in the inventive gel medium displayed a morphology reminiscent of cells in tissues. The fibroblast population continued to grow rapidly until cells filled the lattice of the gel. These fibroblasts appear to be oriented in ordered arrays and exhibited marked biosynthetic activity.

Different concentrations of the inventive peptides in hydrogels were studied. Less than about 50 μg/ml was found to lead to not very efficient cell growth and migration into the gel. Greater than about 200 μg/ml provided results about comparable to that obtained in about 50 μg/ml to about 100 μg/ml concentration range. Different types of cells appear to prefer different inventive peptide concentrations in hydrogels, probably due to differences in receptor densities, but in general about 100 μg/ml will be effective.

Composites of the invention are particularly useful in growing cells, as they include a compound that promotes cell attachment (that is, the compound promotes cell binding in a manner similar to that of collagen, but where the cell binding is enhanced with respect to collagen). Composites of the invention are prepared by associating the cell attachment promoting compound, such as the synthetic peptides earlier described, to a matrix. Because the synthetic peptides define a domain that mimics the cell binding domain of collagen and that has enhanced cell binding with respect to collagen, a number of applications become possible, and matrices useful in forming composites of the invention can take a variety of physical and chemical forms.

For one example, the matrix can be inert, solid and non-porous, such as known and presently used as vessels for cell culture. When such cell culture vessels (petri dishes, flasks and so forth), typically formed of polystyrene or glass, have the cell binding peptides adsorbed or grafted on the surfaces to be exposed to cells, then these treated vessels can be used for tissue culture. The treated vessels have the advantage of anchoring cells with a compound simulating

their natural ligand, unlike other materials now used for coating cell culture dishes. It is well-known that the differentiation and behavior of cells is modulated by their interaction with their natural extracellular matrix. Since the cell binding peptides of this invention are analogues of, or mimic, collagen, they elicit substantially the same response in cells as does collagen itself. The attachment of cells to such treated vessels is markedly greater than on uncoated surfaces or for surfaces coated with gelatin or even when coated with collagen itself.

Another form that may be taken by matrices of this invention is that of soluble polymers. For example, bioreactors are based on the mass culture of cells in various types of media. When cell binding peptides of this invention are carried, or bound to, soluble polymers, then cells can be cultivated in large masses. Because these cultures float in the medium, they are constantly bathed in the medium and have continuous contact with nutrients and additives. Thus, the desired secretory products of the cells can be easily recovered from the medium.

Other suitable matrices for practice of this invention include various polymers and hydrogels. Such composites are useful in constructing templates for repair of soft tissue, for rapid replacement of lost tissue, and for reconstructive and plastic surgery.

Composites of this invention formed with hydrogels as the matrix promote the influx of cells. It is well known that cells differentiate to a greater extent in a three dimensional environment in contact with the surrounding extracellular matrix. Since synthetic peptides of this invention mimic collagen, cells in inventive composites, where the matrices are hydrogels, behave as if the cells are surrounded by extra-cellular matrix and undergo differentiation. Also

for soft tissue repairs, the gels can be injected prior to gelation and then the gel formed in situ.

Composites of the invention can thus be made with resorbable polymers of various kinds, having peptide grafted onto the lattice of the polymeric material. Of course, polymeric supports that are limited in resorbable properties such as hydroxyethyl methacrylate, polymethylmethacrylate, and N-vinylpyrrolidone methylmethacrylate, as a few examples, are also feasible. The composites can then be implanted in the tissue defect. (By "grafting" of peptide so as to be carried by or bound to the matrix is meant the various modes of attachment earlier described.)

Among the known and suitable resorbable hydrogels are combinations of polylactacte and polyglycollate. Compounds of the invention can be covalently bound to such materials during synthesis of the polymers themselves or the polymers can be hydrolyzed such that attachment sites are available by irradiating the polymer or by chemically activating the polymer to generate free radicals. Then conventional techniques for grafting, or immobilizing, peptides onto polymer supports can be utilized to prepare inventive composites. Resorbable hydrogels or polymers so prepared are particularly useful for soft tissue reconstructions. For hard tissue reconstructions or repair (e.g., bone repair) it is desirable to combine such water soluble, or resorbable, polymer species with a bioceramic, such as for example bioglass, aluminum oxide, calcium aluminate, tricalcium phosphate, and hydroxyapatite.

Matrices of the invention can also be porous, and in bead or particulate form. For example, calcium phosphate materials, such as apatite-based ceramics, have been suggested for producing porous tissue implants or prosthesis materials with micropores sufficient to

permit tissue attachment. It is true that hydroxylapatite itself has a limited ability to promote cell attachments; however, inclusion of inventive compounds markedly increases the ability of cells to attach, as is illustrated by Example 2.

EXAMPLE 2

Particulate hydroxylapatite was obtained from CeraMed Corporation of Lakewood, Colorado under the designation "osteograph." Particles were coated by preparing a 100 μg/ml solution of inventive peptide (SEQ ID NO:1) in PBS into which 10 μg of the hydroxylapatite product was dispersed and allowed to sit overnight. The thus coated particles were then incorporated into an agarose gel that either had (a) inventive peptide therein (as described for the Fig. 2 photograph) or had (b) no inventive peptide (as described for the Fig. 1 photograph). Fibroblasts were placed on the surfaces of these gels. Where the particles were coated but the gel did not include inventive peptide (inventive composition (b)), then the cells migrated into the gel to the particles and formed spreading colonies which tended to clump. Where the coated particles were in a gel that also included inventive peptide (inventive composition (a)), there was a very large influx of cells into the gel that organized on and around the coated particles in tissue-like masses.

By contrast, when uncoated particles were included into a prior art agarose gel, a few cells did attach, but at about a ten fold reduction with respect to inventive composition (b).

The osteograph (hydroxylapatite) product, when reconstituted with PBS, is presently used for periodontal repair. As can be readily appreciated, forming an inventive composite with such hydroxylapatite will

provide a significantly improved composition for tissue repair.

EXAMPLE 3

In a manner analogous to that described in Example 2, dense beads of hydroxylapatite (substantially nonporous) have been coated with inventive peptides. Fibroblast cells covered the bead surfaces and have been observed to start "budding" together and forming networks of cells.

In short, substantially all of the known biomaterials presently used or suggested for use as biomaterials can be readily adapted as the matrix component of the inventive composites. (By "biomaterial," is meant the usual definition - "material used for or suitable for use in prostheses that come in direct contact with living tissues.") For example, hydroxylapatite has been used to coat metal implants. Inclusion of the peptides into the coatings should lead to a further ability of cells to attach to such coated implants. Yet further, silicone has been used for applications such as tendon repair or artificial blood vessels and other tissue prostheses, and inclusion or coatings of peptides into or onto such materials should provide benefits.

While composites of the invention are typically used in applications for growing or promoting the growth of cells, another embodiment of this invention is to inhibit the migration of cells. For example, cells that are out of normal regulation processes (e.g., cancer and autoimmune situations) have an increased number of collagen receptors, which can be blocked by the inventive synthetic peptides due to

competitive (and inhibitory) binding with respect to collagen. This aspect of the invention is illustrated by the following T-cell studies. Other implications for the T-cell aspects of this invention include drug delivery to infected T-cells with potential applications in AIDS therapy.

EXAMPLE 4

The interactions of T-lymphocytes with the extracellular matrix play an important role in their differentiation, maturation, and migration. Collagen is a major component of the physiological milieu in which T-cells reside, such as by affecting differentiation and migration behavior.

Mixed populations of peripheral blood CD4 and CD8 lymphocytes showed a strong affinity for substrates coated with rat tail type I collagen. Cultured T-cells were incubated in collagen-coated dishes in serum free medium, in the presence and absence of inventive peptides. The interactions between T-cells and collagen was markedly inhibited by the peptides (SEQ ID NOS. 1, 2, and 3). The maximal rate of T-cell binding was observed in the first 30 minutes in the absence of the inventive peptides. Inhibition was examined at several concentrations and times. Maximal inhibition by the peptides occurred at 30 minutes at 37°C at a concentration of 35 μM. Thus, the molecular site on type I collagen involved in the interaction with T-cells appears to be the same as involved in fibroblast binding.

In conditions such as arthritis, T-cells aggregate. Administration (such as by injection at the affected site) of inventive peptides in a physiologically acceptable solution should prove

efficacious. Administration (intravenous, intradermal, or subcutaneous) in amounts from about 1 to about 200 μK/kg body weight in combination with a pharmaceutically acceptable carrier, such as isotonic saline phosphate buffer solution or the like, should prove therapeutically useful. Pharmaceutically acceptable salts of the inventive peptides with organic and inorganic acids can be formed.

It is to be understood that while the invention has been described above in conjunction with preferred specific embodiments, the description and examples are intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims.

It Is Claimed:

1. A synthetic peptide comprising:

a domain that mimics collagen binding to cells and has enhanced cell binding with respect to collagen, the domain including a core sequence having at least five amino acid residues, two of said core sequence residues being -Ile-Ala- folded in a ß-bend at physiologic conditions, at least two other amino acid residues of the core sequence each flanking the ß-bend, and being in an opposed relationship to each other, Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg (SEQ ID NO:2), Gln-Gly-Ile-Ala-Gly-Gln (SEQ ID NO:3), Gln-Gly-Ile-Ala-Gly-Gln-Arg (SEQ ID NO:4), Phe-Gly-Ile-Ala-Gly-Phe (SEQ ID NO:5), Gly-Ile-Ala-Gly-Gln (SEQ ID NO:6), or Gln-Gly-Ala-Ile-Ala-Gln (SEQ ID NO:7).

2. A composite, useful in growing cells, comprising:

a biomaterial forming a matrix; and,

a compound carried by the matrix, the compound defining a domain that mimics collagen binding to cells and has enhanced cell binding with respect to collagen.

3. The composite as in claim 2 where at least some of the compound is carried within interstices of the matrix, the interstices being of a size sufficient to permit cell growth therein.

4. The composite as in claim 2 wherein at least part of the matrix is substantially water insoluble.

5. The composite as in either claim 2 or 4 wherein at least part of the matrix is a soluble polymer or is a hydrogel.

6. The composite as in claim 2 wherein the compound is attached to the matrix.

7. The composite as in claim 2 wherein the compound is covalently bond to the matrix.

8. The composite as in either claim 2 or 3 wherein at least some of the compound is carried on a surface of the matrix and promotes cell growth thereon.

9. The composite as in claim 2 wherein the compound is selected from the peptides of claim 1.

10. A therapeutic composition comprising a biomaterial and a peptide carried by the biomaterial, the peptide having enhanced cell binding with respect to collagen.

11. The therapeutic composition as in claim 10 wherein the peptide is an amount effective to inhibit cell growth.

12. The therapeutic composition as in claim 10 wherein the biomaterial is of a construction adapted for tissue repair or reconstruction.

13. The therapeutic composition as in claim 12 wherein the biomaterial includes an apatite-based ceramic.

14. The therapeutic composition as in claim 10 wherein the biomaterial includes a hydrogel.

15. The composition as in claim 10 wherein the peptide is selected from the peptides of claim 1.

FIG. 1. PRIOR ART

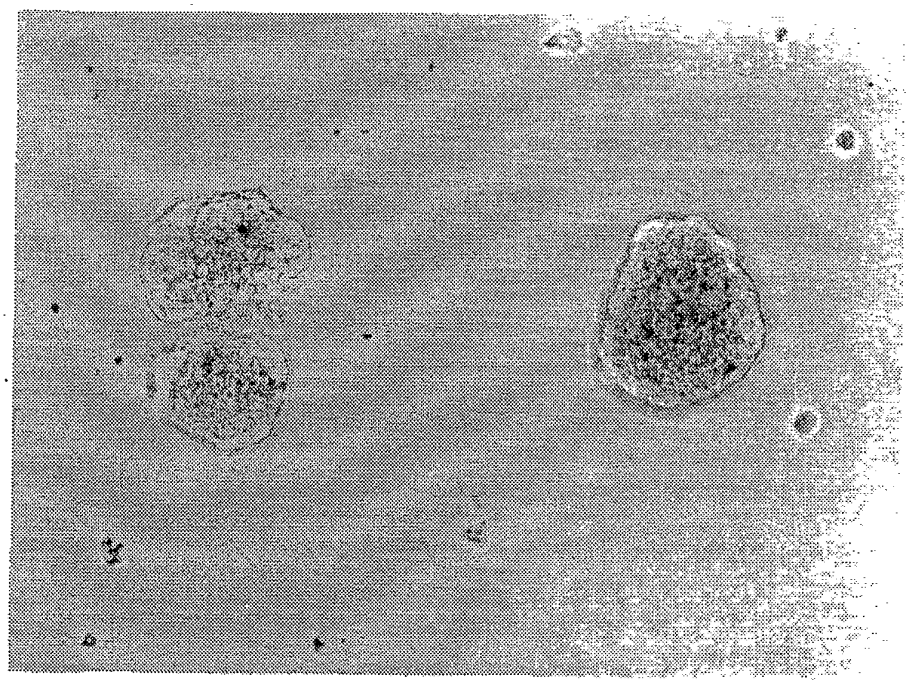

FIG. 2.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10420

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/00, 37/02; CO7K 5/00, 7/00, 15/00, 17/00
US CL :530/329, 330; 514/16, 17, 18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/329, 330; 514/16, 17, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS TEXT SEARCH, BIOSIS, CA, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TETRAHEDRON LETTERS, VOL. 29, NO.34, ISSUED 1988, GREEN ET AL,"APPLICATION OF THE NG-(2,2,5,7,8-PENTAMETHYLCHROMAN-6-SULPHONYL) DERIVATIVE OF FMOC-ARGININE TO PEPTIDE SYNTHESIS",PAGES 4341-4344, SEE ENTIRE DOCUMENT.	1-15
Y	THE JOURNAL OF BIOLOGICAL CHEMISTRY, VOL. 262, NO. 13, ISSUED 05 MAY 1987, FIELDS ET AL, "SEQUENCE SPECIFICITY OF HUMAN SKIN FIBROBLAST COLLAGENASE EVIDENCE FOR THE ROLE OF COLLAGEN STRUCTURE IN DETERMINING THE COLLAGENASE CLEAVAGE SITE", PAGES 6221-6226, SEE ENTIRE DOCUMENT.	1-15
Y	BIOCHEMISTRY, VOL. 24, ISSUED 1985, WEINGARTEN ET AL, "SYNTHETIC SUBSTRATES OF VERTEBRATE COLLAGENASE", PAGES 6730-6734, SEE ENTIRE DOCUMENT.	1-15
Y	BIOCHIMICA ET BIOPHYSICA ACTA, VOL. 445, ISSUED 1976, NAGAI ET AL, "SUBSTRATE SPECIFICITY OF VERTEBRATE COLLAGENASE", PAGES 521-524, SEE ENTIRE DOCUMENT.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search
23 FEBRUARY 1992

Date of mailing of the international search report
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