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Self-complementary oligopeptide matrices support mammalian cell attachment

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A new class of ionic self-complementary oligopeptides is described, two members of which have been designated RAD16 and EAK16. These oligopeptides consist of regular repeats of alternating ionic hydrophilic and hydrophobic amino acids and associate to form stable β-sheet structures in water. The addition of buffers containing millimolar amounts of monovalent salts or the transfer of a peptide solution into physiological solutions results in the spontaneous assembly of the oligopeptides into a stable, macroscopic membranous matrix. The matrix is composed of ordered filaments which form porous enclosures. A variety of mammalian cell types are able to attach to both RAD16 and EAK16 membranous matrices. These matrices provide a novel experimental system for analysing mechanisms of *in vitro* cell attachment and may have applications in *in vivo* studies of tissue regeneration, tissue transplantation and wound healing.

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The discovery and design of novel biomaterials have become increasingly important for advanced tissue engineering and controlled drug delivery systems[1,2]. Several new biomaterials, consisting of copolymers of synthetic organic polymers and proteins, have been introduced in the last decade. These include hydrogels consisting of a copolymer of poly(vinyl alcohol) and collagen[3], a copolymer of poly-L-lactide/poly-ε-caprolactone for nerve regeneration[4] and collagen-glycosaminoglycan, which induces partial regeneration of adult mammalian skin[5]. Two recent reports show that copolymers containing either a poly(L-lactic acid and L-lysine) backbone[6] or a polyurethane backbone[7], linked with oligopeptide side-chains containing the arginine–glycine–aspartate (RGD) cell adhesion motif, can serve as a ligand for cell attachment[6,7]. The RGD motif is an important ligand for some members of the integrin family of cell adhesion receptors[8,9]. The combined use of synthetic polymer chemistry and oligopeptides corresponding to biological motifs represents a growing trend and a significant advance in the development of biomaterials.

We have recently described a new class of ionic self-complementary oligopeptide[10,11]. These oligopeptides consist of alternating hydrophilic and hydrophobic amino acids. When the oligopeptides form a β-sheet, they have two surfaces, one polar surface with charged ionic side chains, the other a non-polar surface with alanines. These ionic side chains are complementary to one another, i.e. the positively charged and negatively charged amino acid residues can form ionic pairs. A defining characteristic of this family of self-complementary oligopeptides is their ability to spontaneously assemble to form stable macroscopic matrices in the presence of monovalent cations. One member of this family, EAK16 (AEAEAKAKAEAEAKAK), was originally found as tandem repeats in the yeast protein, zuotin, which had been characterized as a left-handed Z-DNA binding protein[12]. EAK16 was systematically altered to design another oligopeptide, RAD16, in which positively charged arginines substitute for lysines and negatively charged aspartic acids substitute for glutamic acids.

All of these oligopeptides consist of L-amino acids, and their degradation will yield normal amino acids. Because these oligopeptides have primary sequences that consist of alternating hydrophobic and hydrophilic side-chains, they are highly soluble in pure water and have a tendency to form an unusually stable β-sheet structure[10,11]. Under the appropriate conditions, the β-sheets formed from such oligopeptides can spontaneously assemble to form large, regular macroscopic structures; this assembly is facilitated by ionic side-chain interactions in addition to conventional β-sheet

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backbone hydrogen bonding. Once assembled, these matrices are chemically and physically stable under physiological conditions. They are resistant to heat, to many chemical denaturation agents, and to degradation by proteolytic enzymes *in vitro*[10,11]. To explore this field further, many other members of the family can be synthesized in large quantities by an automated peptide synthesizer and tailored *de novo* for particular applications.

Here, we show that these oligopeptide matrices can support the attachment of a variety of cultured mammalian cells. We also show that cell adhesion to RAD16, which contains two RAD motifs, occurs independently of cell adhesion mechanisms involving the RGD motif, to which some integrins bind.

MATERIALS AND METHODS

Oligopeptides

Both EAK16 and RAD16, as well as GRGDSP, were chemically synthesized using solid-state phase peptide synthesis technology by an Applied Biosystems Model 430A peptide synthesizer and HPLC purified as described in detail in Zhang *et al.*[11]. The oligopeptide sequences are as follows:

$$\begin{array}{ll} & \overset{-}{} \overset{-}{} \overset{+}{} \overset{+}{} \overset{-}{} \overset{-}{} \overset{+}{} \overset{+}{} \\ \text{EAK16,} & \text{AcN–AEAEAKAKAEAEAKAK–CNH}_2 \end{array}$$

$$\begin{array}{ll} & \overset{+}{} \overset{+}{} \overset{-}{} \overset{-}{} \overset{+}{} \overset{+}{} \overset{-}{} \overset{-}{} \\ \text{RAD16,} & \text{AcN–RARADADARARADADA–CNH}_2 \end{array}$$

$$\begin{array}{l} \overset{+}{} \overset{-}{} \\ \text{GRGDSP} \end{array}$$

where A is alanine; E is glutamic acid; K is lysine; R is arginine; D is aspartic acid; G is glycine; S is serine; P is proline. N- and C- refer to the N- and C-termini of the peptides, − and + refer to the positively and negatively charged residues. Oligopeptides were blocked on both the N- and C-termini.

Cell cultures

The HIT-T15 cells (hamster pancreatic β cells) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were grown in Ham's F-12 (Gibco, Grand Island, NY, USA), 2.5% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 10% horse serum (HS; Gibco, Grand Island, NY, USA). Normal human epidermal keratinocytes (NHEK) were purchased from Clonetics (San Diego, CA, USA), and were grown in Keratinocyte Growth Medium (KGM; Clonetics, San Diego, CA, USA). Other cell lines were from the collection of R.O. Hynes and were grown in the following media: CEF (chicken embryo fibroblast), HFF (human foreskin fibroblast), HepG2 (human hepatocellular carcinoma), MG63 (human osteosarcoma), and Hela cells (human epitheloid carcinoma) and HEK293 cells (human embryonic kidney) in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand Island, NY, USA), 10% FBS; NIH 3T3 cells (mouse embryo) in DMEM, 10% calf serum (CS; Sigma, St. Louis, MO, USA); CHO (Chinese hamster ovary) cells in minimal essential medium α (MEMα; Gibco, Grand Island, NY, USA), 10% FBS; PC12 (rat adrenal pheochromocytoma) cells in RPMI (Gibco, Grand Island, NY, USA), 10% horse serum, 5% FBS; SH-SY5Y (human neuroblastoma cells, gift of J. Biedler, Memorial Sloan-Kettering Cancer Centre, New York, USA), in DMEM, 10% FBS, 2 mM glutamine; mouse granule cells (8-day mouse cerebellum, gift from M. Didier, McLean Hospital/Harvard Medical School, Boston, MA, USA) in 1:1 DMEM:F12, 5% FBS, 5% HS, 2 mM glutamine, 5 mM glucose, 10 mM HEPES, 3 mM $NaHCO_3$.

Circular dichroism of RAD16 and scanning electron microscopy (SEM) of oligopeptide membrane

The circular dichroism (CD) measurements of RAD16 were carried out on an Aviv model 60DS spectropolarimeter using program 60HDS for data processing as described previously[10,11]. The oligopeptide matrices were prepared for SEM by incubating the membranes in 5% glutaraldehyde at 4°C for 30 min, followed by sequential dehydration steps with 10, 20, 50, 70, 90 and 100% ethanol for 5 min each. The sample was placed in pressurized liquid CO_2/syphon for 1 h. The sample was then coated with gold particles, mounted on a grid and examined between ×400 and ×20 000 magnification.

Cell attachment to oligopeptide-based matrices

Macroscopic membranous materials were self-assembled by pipetting 10–50 μl of aqueous oligopeptide solution (1–2 mg ml^{-1}) into 0.5–1 ml of the appropriate cell culture media in 6- or 24-well tissue culture plates (Beckton-Dickinson, Lincoln Park, NJ, USA). For coating the plates with oligopeptides, EAK16 and RAD16 solutions (25 μg per well, 15 mm diameter) were added to the plates and dried overnight at 60°C. For plating of cells onto oligopeptide membranes, cells were removed from tissue culture dishes with versene, rinsed with phosphate-buffered saline (PBS), and plated at subconfluent densities into the wells containing the membranes. In most cases, in order to separate membrane-attached cells from unattached cells, after cell attachment (3–4 h following introduction of the cells), the membranes were transferred using a plastic pipette tip with a 0.4–0.5 cm bore to new culture wells containing fresh medium. Cell cultures were incubated at 37°C, 5% CO_2. For long-term culture of cells on oligopeptide membranes, fresh medium was added to wells every 3–4 days.

Inhibition of integrin-mediated cell attachment by a water-soluble oligopeptide containing RGD was performed as follows: MG63 or HFF cells were washed twice with PBS and resuspended in PBS containing the GRGDSP peptide (8 mM), then preincubated in 1.5 ml Eppendorf tubes at 37°C with gentle mixing for 30 min. Preincubated cells were then divided into equal parts in serum-free medium and added to 24-well plates either coated with 20 μg ml^{-1} of human plasma fibronectin or containing the isobuoyant membrane formed from the self-complementary oligopeptide RAD16. The cells were incubated at 37°C

for 30 min to allow attachment, then placed on a shaker with a rotation speed of 150 rpm at 37°C for 10 min. The cells were then further incubated for approximately 3 h, and then visually assayed qualitatively by three individuals over a period of 2 weeks. Criteria noted for quality of cell attachment include cell density on membrane matrices and ability of cells to remain attached when agitated. The membranous matrices with cells attached were photographed using a phase contrast microscope.

The viability of the cells that attached to the oligopeptide matrices was assessed by their morphological appearances using the phase-bright viability index as described by Baughman *et al.*[13]. The phase-bright viability index is conventionally used to describe cells in culture by phase contrast microscopy[13–15]. Viable cells are phase-bright with smooth membrane surfaces and round nuclei, whereas dying cells exhibit irregular membrane surfaces, reduced cytoplasmic volume and condensed nuclei, thus resulting in phase-dark cells. These morphological characteristics correlate with other indices of cell viability such as ultrastructural analysis by electron microscopy and the release of cellular enzymes into the medium following cell lysis.

RESULTS

Structural features of oligopeptides and matrices

Both EAK16 and RAD16 are members of the ionic self-complementary oligopeptide family. The CD spectra of both RAD16 and EAK16 in water (*Figure 1*) reveal an ellipticity with a minimum at 218 nm and a maximum at or near 194 nm, indicating a β-sheet structure. The β-sheet content of RAD16 and EAK16 is calculated to be approximately 100% using ellipticity at 218 nm[16]. Since all β-sheets have a slightly right-handed twist, the different ellipticity near 194 nm between RAD16 and EAK16 probably reflects a difference in the twists (*Figure 1*). When the aqueous oligopeptide was

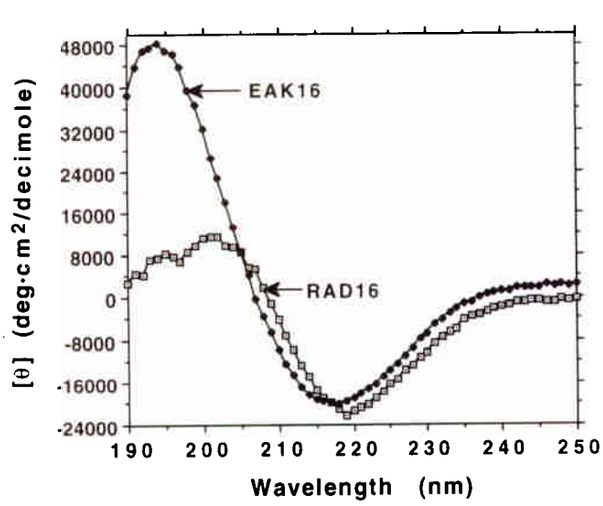

Figure 1 Circular dichroism (CD) spectra of RAD16 and EAK16 in water. RAD16 (10 μM) and EAK16 (10 μM) were used for the measurement. These are characteristic β-sheet CD spectra with a minimum ellipticity at 218 nm and a maximum at 194 nm. The β-sheets of RAD16 and EAK16 may have different twists as suggested by the different ellipticity at 194 nm.

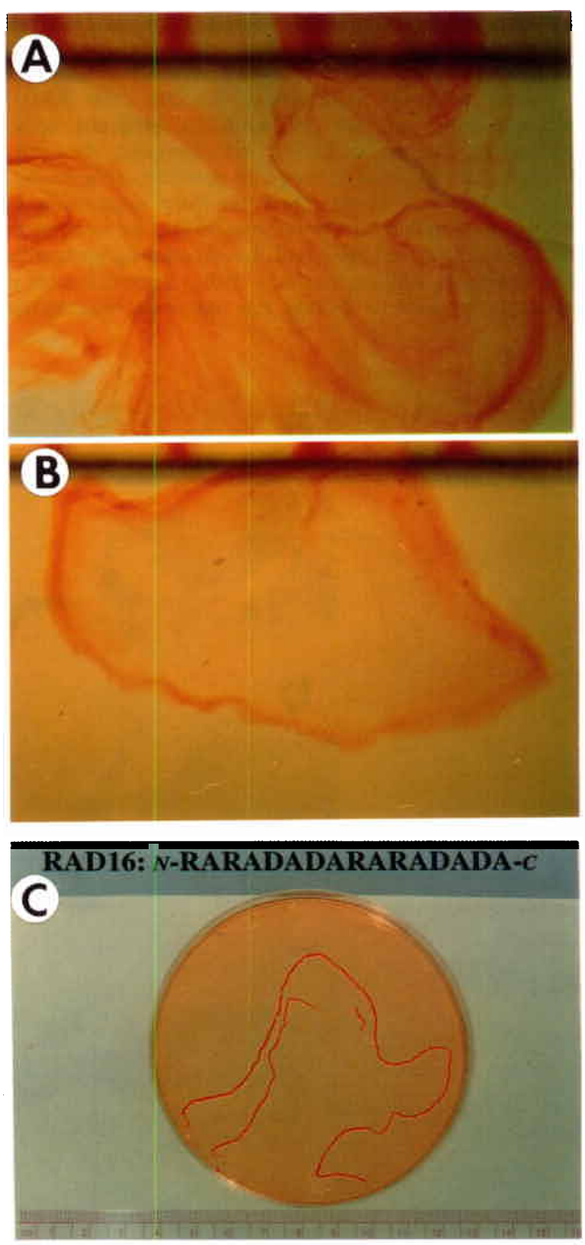

Figure 2 Photographs of the oligopeptide matrices. The membranes were stained with Congo Red (10 μg ml^{-1}); **A**, EAK16 and **B**, RAD16, which stains less well. The divisions at the top of the photograph equal 1 mm. Areas that appear to stain more strongly in **A** are due to folding of the membrane, which reduces transparency. **C**, The RAD16 oligopeptide-based biomaterial was processed to form fibres. The liquid peptide dissolved in water was injected into a PBS solution through a fine pipette tip of approximately 0.1 mm diameter. A strand more than 15 cm in length has been produced. A scale in centimetres is shown at the bottom. EAK16 can be produced in a similar manner.

introduced into either a solution containing monovalent salt, a physiological buffer, or cell culture media, the monomeric oligopeptide spontaneously assembled to form a stable, membranous material. The oligopeptide membranes could be stained with Congo Red dye (*Figure 2*). Congo Red preferentially stains β-sheet structures in proteins and is widely used as a histological stain to detect deposition of amyloid plaques in tissues[17].

Using different conditions, EAK16 and RAD16 oligopeptides can be produced in different stable geometric shapes, including membranous structures (*Figure 2A* and *B*) or thread-like materials (*Figure 2C*). The dimensions of the materials vary depending on the concentrations of the oligopeptides, the concentration of monovalent salts and the processing apparatus. For example, when a cannula with a bore diameter of >0.2 mm is used to deliver the materials, membrane-like matrices were formed (*Figure 2A* and *B*). The membranes can be extended for several square centimetres when a large amount of material is used. Thread-like structures are formed if a cannula with a bore diameter <0.1 mm and a slow delivery into salt solution are employed. *Figure 2C* shows a thread-like structure formed by oligopeptide RAD16, which is greater than 15 cm in length. SEM of EAK16 revealed a fibrous structure consisting of interwoven filaments with diameters of 10–20 nm and porous enclosures of about 50–100 nm diameter (*Figure 3*).

Cell attachment to EAK16 and RAD16 matrices

Many extracellular matrix molecules, including fibronectin, contain the triplet RGD that is recognized by integrins as a binding motif for cell adhesion[8,9]. Interestingly, the RGD in cytotactin/tenascin is not

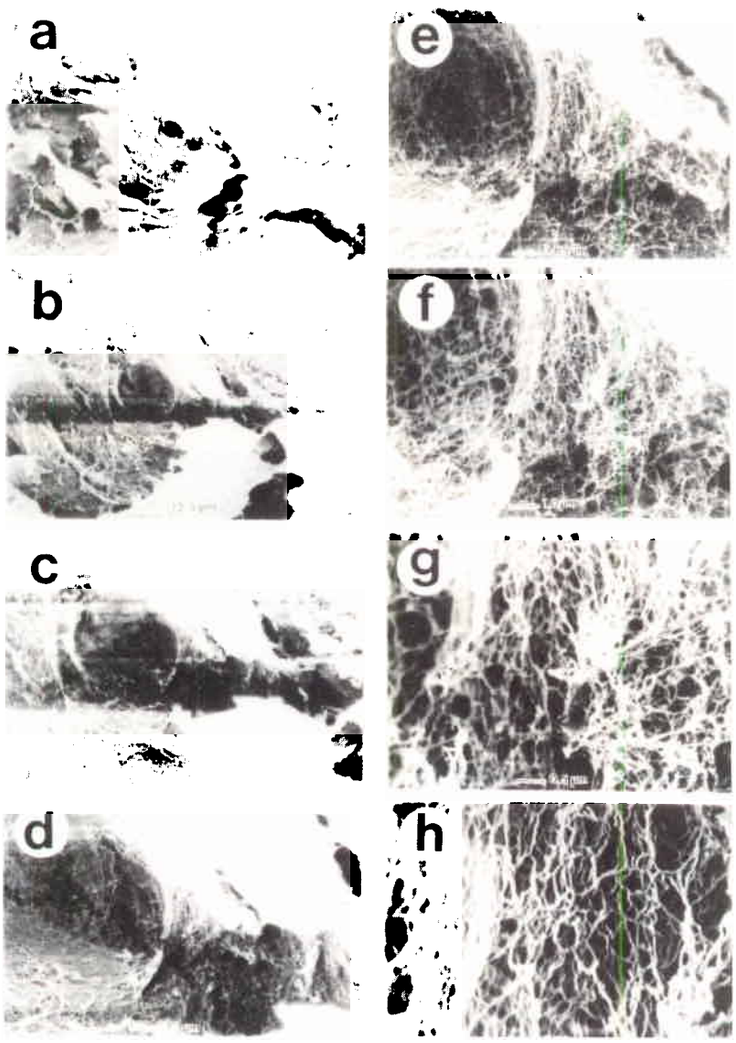

Figure 3 Serial photography of scanning electron microscopy (SEM) of EAK16. The photographs were taken at the following original magnifications: **a**, ×400; **b**, ×800; **c**, ×1600; **d**, ×3000; **e**, ×6000; **f**, ×10 000; **g**, ×15 000; and **h**, ×20 000. The diameter of the filaments is approximately 10–20 nm and the enclosures between the fibres are approximately 50–100 nm. A reference bar measured in μm is included in each photo.

Table 1 Cells tested for attachment to the oligopeptide EAK16 and RAD16 matrices*

Cell type	Cell line
Mouse fibroblast	NIH-3T3
Chicken embryo fibroblast	CEF
Chinese hamster ovary	CHO
Human cervical carcinoma	Hela
Human osteosarcoma	MG63
Human hepatocellular carcinoma	HepG2
Hamster pancreas	HIT-T15
Human embryonic kidney	HEK293
Human neuroblastoma†	SH-SY5Y
Rat pheochromocytoma†	PC12
Mouse cerebellum granule cells†‡	
Mouse hippocampal cells†‡	
Human foreskin fibroblast‡	
Human epidermal keratinocytes‡	

*Various cell types attached to the oligopeptide matrices. Visual assessment of cell attachment was performed using phase contrast microscopy over a period of 2 weeks.
†Neuronal cells.
‡Cells derived from primary cultures.

completely conserved in all species; for example, an RAD motif, in which alanine (A) substitutes for glycine (G), occurs in cytotactin of pig[18]. Furthermore, a recent report has shown that a glycine in the RGD motif can be substituted by an alanine without significant loss of cell attachment activity, while substituting valine for glycine in the RGD reduced cell attachment[19]. Therefore, it seemed possible that oligopeptide matrices containing RAD motifs, such as the RAD16 membranes, could support cell attachment.

To determine whether cultured cells could attach to RAD16 or EAK16 matrices, cells were applied to the membrane matrices and incubated for 3–4 h before visual estimates of cell attachment were performed. A number of cell types from established cell lines and cells derived from primary cultures were tested, and it was found that they attached to both oligopeptide matrices (*Table 1*). The cells remained well attached even after the membrane matrices were transferred to fresh culture wells and agitated on a shaker at 150 rpm for 10 min.

Figure 4 shows attachment of MG63 osteosarcoma cells (*Figure 4A*) to RAD16 matrices. We also tested the effect of RGD-mediated attachment. Cells attached on plasic tissue culture plates coated with fibronectin (*Figure 4B*) or to the RAD16 membrane (*Figure 4C*). As expected, MG63 cells spread very well on the rigid surface of the fibronectin-coated plastic and displayed extended processes from the cell bodies (*Figure 4B*). We also tested cell attachment to EAK16- and RAD16-coated plastic plates. Peptide coating of wells was confirmed by using Congo Red staining. EAK16 and RAD16 form a thin layer, which sometimes peels away from the plastic. Cells that attach to tissue culture plastic coated with oligopeptide exhibit a flat, spread morphology similar to that seen on fibronectin-coated plastic. In contrast, the same cells retained a round shape and did not spread on the malleable RAD16 membranes (*Figure 4C*). Cells that attached to portions of the peptide matrix that peeled from the plastic exhibited a round morphology, similar to the morphology seen on the oligopeptide membranes. Similar morphologies were observed for most cell types tested (not shown). These observations are consistent with other systems in which various cell types assume flattened, spread morphologies on rigid substrates, such as plastic or plastic coated with extracellular matrix proteins, but are more cuboidal or rounded on malleable extracellular matrices, such as gels of type I collagen or basement membrane Matrigel™ [20,21]. It is reasonable that the malleable oligopeptide membranes, like extracellular matrix gels, can yield to cytoskeletal tensions, resulting in the cells assuming a rounded morphology, while rigid substrates such as plastic cannot[22]. Our results are consistent with previous observations that cells retain more rounded morphologies on malleable materials[20–23].

To ascertain whether cell attachment to the RAD16 membrane is mediated by an RGD-dependent mechanism of cell adhesion, cells were preincubated with water-soluble oligopeptides containing the RGD sequence, then transferred either to RAD16 membranes or to fibronectin-coated plastic as a control. As expected, preincubation of MG63 cells with the RGD peptide (8 mM) completely inhibited attachment to fibronectin (*Figure 4D*). However, the same cells were still able to attach to the RAD16 membrane (*Figure 4E*). Similar results were obtained for HFF (not shown). These observations suggest that RGD-mediated adhesion by integrins is not critical for the initial attachment of cells to the RAD16 membrane.

We attempted to produce antibodies using RAD16 and EAK16 oligopeptides. However, it is interesting to observe that when these oligopeptides were either alone or conjugated with other proteins and injected into rabbits, they did not elicit a detectable immune response, and no significant titres of antibodies were obtained. We speculate that the lack of immunogenicity may be partly due to sequence similarities and distributions of charged amino acids between these oligopeptides and a segment found in intermediate neural filament H, L and M proteins[24]. This lack of immunogenicity is likely to be important so as to reduce immune rejection if we develop these oligopeptides for tissue engineering.

DISCUSSION

A proposed structural model for oligopeptide matrix formation

Both EAK16 and RAD16 belong to a subtype of the ionic self-complementary oligopeptide family, termed modulus two, where two positively charged arginines on the ionic side of the β-sheet are followed by two negatively charged aspartic acids in the case of RAD16 (*Figure 5A*[25,26]), or two negatively charged glutamic acids are followed by two positively charged lysines in the case of EAK16 (*Figure 5B*). In general, short oligopeptides, such as 16mers, are not considered as building blocks for biomaterials, and most oligopeptides do not form regular or stable structures. In contrast, the oligopeptide EAK16 maintains unusually stable β-sheet structures in water even under extreme conditions[11]. Furthermore, they spontaneously

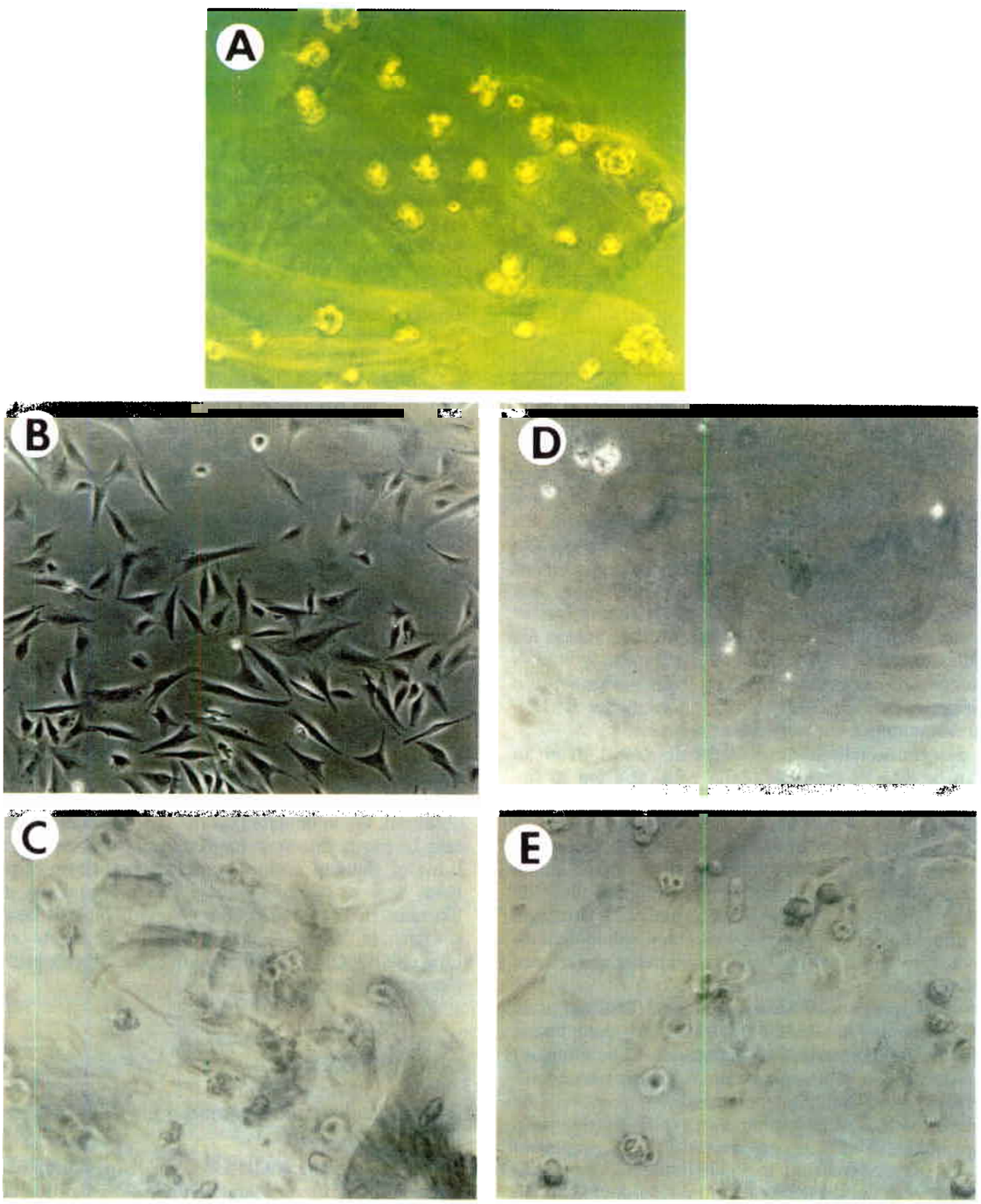

Figure 4 Cell attachment to RAD16 matrices. Human MG63 osteosarcoma cells attached to RAD16 matrix, **A**. Cells in panels **C–F** were incubated for 30 min in the absence, **B**, **C** or presence, **D**, **E** of an oligopeptide GRGDSP (8 mM) which inhibits RGD-mediated cell adhesion. **B** MG63 cells were plated on tissue culture plastic coated with 20 $\mu g\,ml^{-1}$ human plasma fibronectin, or **C** the RAD16 membrane matrix. Photographs were taken approximately 4 h after plating. In panels **D** and **E**, the same cells were preincubated with GRGDSP peptide and then transferred either to a fibronectin-coated well **D** or to the RAD16 membrane **E**. GRGDSP inhibits cell attachment to fibronectin, but not to the RAD16 membrane. In panel **E** some cells are seen floating above the focal plane. Panels **C** and **E** show the cells attached to the membrane with folded membrane segments visible; some portions of RAD16 membrane lie outside of the focal plane.

assemble to form stable macroscopic membranes upon addition of monovalent salts in physiological conditions[10]. We also designed and synthesized *de novo* the oligopeptide RAD16, where both glutamic acid (E) and lysine (K) of the original EAK16 were substituted with aspartic acid (D) and arginine (R),

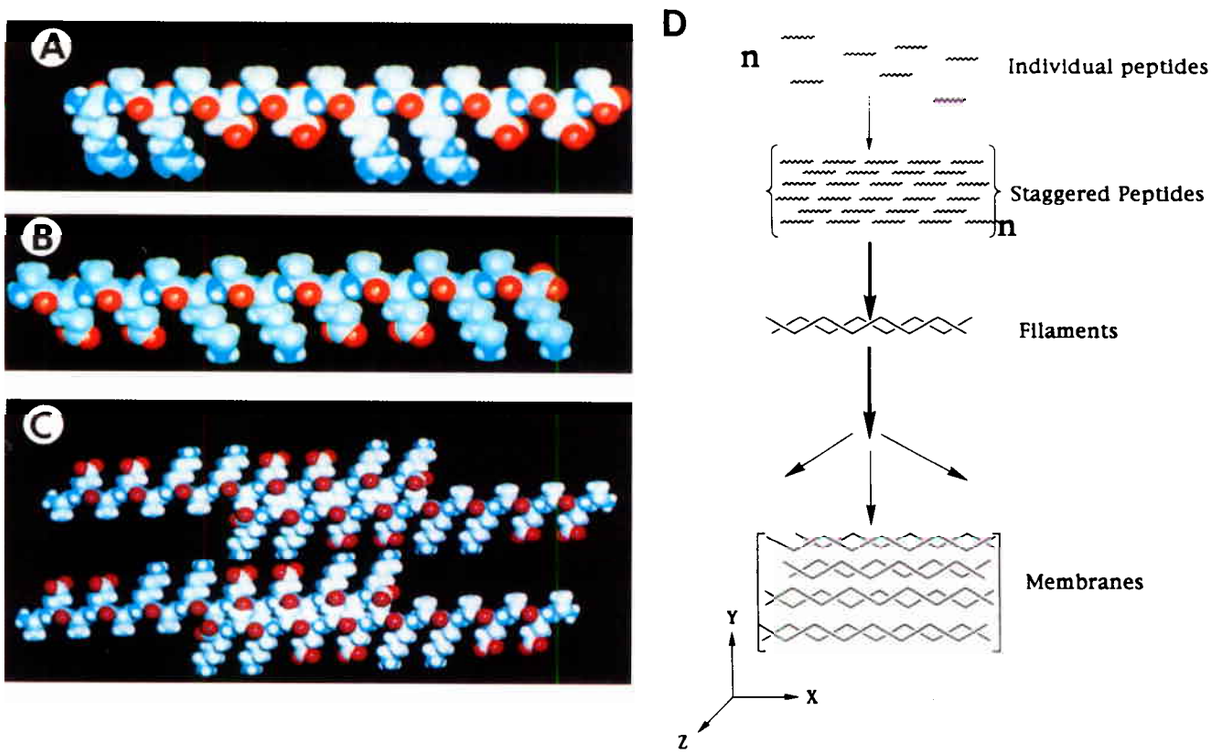

Figure 5 A proposed model of sequential events which could lead to assembly of macroscopic matrices. Molecular models of the extended β-strand structures of individual molecules are shown for RAD16 **A** and EAK16 **B**. Carbons are coloured light green, nitrogens are blue, oxygens are red and hydrogens are white. The distance between the charged side-chains along the backbone is approximately 0.69 nm[25,26]; the methyl groups of alanines are found on one side of the sheet. Conventional β-sheet hydrogen bond formation between the oxygens and hydrogens on the nitrogens of the peptide backbones is perpendicular to the page. **C** A proposed staggered assembly of molecular models for EAK16. The complementary ionic bonds and hydrophobic alanines are shown. Although an antiparallel β-sheet is illustrated, a parallel β-sheet model is also possible. **D** One of the possible pathways of matrix formation. X, Y, and Z indicate three dimensions of the materials. Geometric shapes other than membrane can also be produced (*Figure 2C*), as suggested by the diverging thin arrows.

respectively. The charge orientation of RAD16 with respect to EAK16 is also reversed (see the Materials and Methods section). In addition to ionic interactions, the hydrophobic interactions of RAD16 and EAK16 in the proposed molecular structure resemble the molecular architecture of silk[27–29], where alanine–alanine and glycine–glycine interact very closely. In silk fibroin, the protein has a fully-extended backbone that forms β-sheets with staggered alanines and other residues[28,29]. However, the molecular weight of silk fibroin is about 200 times greater than that of the self-complementary oligopeptides RAD16 and EAK16. Therefore, silk fibroin has more repeats than these oligopeptides. It is interesting that the collective interactions of the ionic bond surface and hydrophobic surface of the oligopeptides make it possible to form macroscopic matrices.

We have proposed a molecular model to interpret the formation of macroscopic matrices by EAK16 based on structural data and experimental observations (*Figure 5C*). Several features of the individual oligopeptides contribute to the formation and stability of the structure: (i) oligopeptides form intermolecular hydrogen bonds in conventional β-sheets on the peptide backbones, (ii) the side-chains of positively and negatively charged residues form intermolecular ionic bonds, (iii) methyl groups of alanine form hydrophobic β-sheet interactions, and (iv) the individual oligopeptides may be staggered. The features (ii)–(iv) are illustrated in *Figure 5C*. This proposed structural model correlates well with our current experimental analysis and serves as a working hypothesis and a framework for future experimental designs. It is not yet clear where the monovalent ions are located in the matrices. It is possible that the ions may coordinate the charged residues in a higher order of geometry, thereby promoting formation of the supramolecular structures. Further accumulation of data may revise some of the details in the model. One possible pathway for matrix assembly is schematically illustrated in *Figure 5D*. In this model, many individual extended oligopeptides associate to form staggered β-sheets, which can then form filaments and further self-assemble to form macroscopic membranes.

We have previously investigated several β-sheet-forming oligopeptides that do not have the property of self-complementarity[10,11]. We also studied oligopeptides that formed α-helices and random-coils rather than β-sheets. Although these oligopeptides have similar composition and length, they do not form

macroscopic structures. Thus, in addition to self-complementarity, other factors are likely to be important for the formation of matrix structures, such as the peptide length, the degree of intermolecular interaction and the ability to form staggered arrays.

Cell attachment to the oligopeptide matrices

The amino acid sequence RGD, when present in certain extracellular matrix molecules and in synthetic oligopeptides, acts as a ligand for cell adhesion receptors such as integrins[8,9]. One study comparing oligopeptides containing either RGD or RAD showed that there are no significant differences in the cell attachment activity to either motif[19]. Since RAD16 contains two copies of an RAD motif, we compared the attachment of mammalian cells to RAD16 and EAK16 oligopeptide matrices. Cell attachment to both EAK16 and RAD16 membranes is comparable for a number of cell types (not shown here). Furthermore, water-soluble oligopeptides containing the RGD motif, used at concentrations that blocked adhesion of MG63 cells to fibronectin, do not inhibit MG63 cell attachment to the RAD16 membrane. These results suggest that RGD-binding integrins are not required for cell attachment to RAD16. It is possible that, since within both EAK16 and RAD16 every other amino acid is a charged residue, interactions between charged residues and cell surface components play a role in non-integrin-mediated cell attachment to the oligopeptide membranes. Future experiments using different oligopeptides with reduced charges will address this hypothesis.

The RAD16 and EAK16 oligopeptide membranes were found to support attachment of diverse cell types, including fibroblasts and keratinocytes. However, cells did not spread on the malleable membranes but remained rounded. In contrast, the same cells spread when they were attached to the rigid plastic surface of the tissue culture plate. Rounded cells have also been observed when other malleable matrices were used as substrates for cell attachment. Indeed, malleable extracellular matrices have been shown not only to support healthy cell cultures but also to maintain differentiated morphologies and functions for a number of cell types[22], including hepatocyte-derived cells[21] and mammary epithelial cells[23]. It will be interesting to determine whether the oligopeptide matrices can influence differentiation of attached cells. Moreover, the cell densities on the oligopeptide matrices appeared to increase over time. We observed that the confluence of cells was approximately 3–5% at the time of introduction to the membrane matrices, and the confluence was approximately 70–80% during a two week incubation. Although some variation in the proportion of cells that attached to the matrices is observed between various cell types, these differences have not been quantitatively addressed in this study.

We have described a novel class of self-complementary β-sheet oligopeptides that self-assemble to form biologically compatible matrices under mild aqueous conditions triggered by the addition of monovalent salts. These matrices are able to support mammalian cell attachment for prolonged periods.

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