Design and function of novel osteoblast-adhesive peptides for chemical modification of biomaterials

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Abstract: Proactive, ''next generation'' dental/orthopedic biomaterials must be designed rationally to elicit specific, timely, and desirable responses from surrounding cells/tissues; for example, such biomaterials should support and enhance osteoblast adhesion (a crucial function for anchorage-dependent cells). In the past, integrin-binding peptides have been immobilized on substrates to partially control osteoblast adhesion; the present study focused on the design, synthesis, and bioactivity of the novel peptide sequence Lys-Arg-Ser-Arg that selectively enhances heparan sulfate-mediated osteoblast adhesion mechanisms. Osteoblast, but not endothelial cell or fibroblast, adhesion was enhanced significantly ($p < 0.05$) on substrates modified with Lys-Arg-Ser-Arg peptides, indicating that these peptides may be osteoblast- or bone cell specific. Blocking osteoblast cell-membrane receptors with various concentrations of soluble Arg-Gly-Asp-Ser peptides did not inhibit subsequent cell adhesion on substrates modified with Lys-Arg-Ser-Arg peptides, providing evidence that osteoblasts interact with Arg-Gly-Asp-Ser and with Lys-Arg-Ser-Arg peptides via distinct (i.e., integrin- and proteoglycan-mediated) mechanisms, each uniquely necessary for osteoblast adhesion. The present study constitutes an example of rational design/selection of bioactive peptides, confirms that osteoblast adhesion to substrates can be controlled selectively and significantly by immobilized peptides, and elucidates criteria and strategies for the design of proactive dental/orthopedic implant biomaterials. © 1998 John Wiley & Sons, Inc. *J Biomed Mater Res,* **40,** 371–377, 1998.

Key words: osteoblast; adhesion; peptide; integrin; heparan sulfate

INTRODUCTION

Proactive, ''next generation'' dental/orthopedic biomaterials elicit specific, timely, and desirable responses from surrounding cells and tissues. Specifically, such proactive materials must be designed intelligently to encourage appropriate functions of osteoblasts (the bone-forming cells). Because adhesion is a crucial process that must occur before subsequent osteoblast (an anchorage-dependent cell) functions (such as proliferation, migration, production, and deposition of mineralized matrix) can take place, proactive biomaterials should be designed to support and enhance osteoblast adhesion.

Osteoblasts adhere to Arginine (Arg)—Glycine (Gly)—Aspartic Acid (Asp), or RGD peptide sequences via cell-membrane integrin receptors;[1–3] however, since the RGD-mediated mechanism does not completely account for osteoblast adhesion, other mechanisms also contribute to the adhesion process.[1,3] For example, cell-membrane heparan sulfate proteoglycan interactions with heparin-binding sites on extracellular matrix proteins (such as fibronectin and collagen) may also regulate osteoblast adhesion. A number of reports support this hypothesis: heparan sulfate was detected immunohistochemically on the membranes of osteoblasts attached to bone matrix;[4] blocking the heparin-binding sites of fibronectin with Platelet Factor IV inhibited approximately 45% of the subsequent osteoblast adhesion to this fibronectin;[2] heparan sulfate completely inhibited human, bone-derived, osteoblast-like cell attachment to the heparin-binding region of fibronectin.[3]

While minimal, active, integrin-binding peptides (for example, the RGD sequence) have been successfully immobilized on substrates to partially control osteoblast adhesion,[5] minimal, bioactive, peptide sequences that enhance proteoglycan-mediated osteoblast adhesion had not been identified before the present study. Elucidating such peptide sequences is of importance to the field of bone-cell/tissue engineering as well as to the design of proactive biomaterials: a

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proactive dental/orthopedic biomaterial designed to maximize osteoblast adhesion should enhance heparan sulfate-mediated, as well as integrin-mediated, adhesive mechanisms. Therefore, the present study focused on providing and illustrating strategies for the rational design/selection of bioactive (e.g., adhesive) peptides as well as on elucidating the cell-adhesive properties of such peptides immobilized on model biomaterial surfaces.

MATERIALS AND METHODS

Cells

While neonatal rat calvarial osteoblasts were the main cell line used in the present study, bovine pulmonary artery endothelial cells and rat skin fibroblasts were used for comparative purposes. Endothelial cells (cell line CCL-209) and fibroblasts (cell line CRL-1213) were purchased from and characterized by the American Type Culture Collection. Endothelial cells [in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% calf serum] and fibroblasts (in DMEM supplemented with 10% calf serum) were maintained under standard culture conditions, that is, in a sterile, humidified, 37°C, 5% CO_2/95% air environment. Endothelial cells at population numbers 21–24 and fibroblasts at population numbers 13–17 were used in the adhesion experiments of the present study.

The neonatal rat calvarial osteoblasts used in this study were isolated via sequential enzymatic digestion following published methods[6,7] and cultured in DMEM supplemented with 10% calf serum under standard culture conditions. The osteoblast phenotype was confirmed by a number of tests, including alkaline phosphatase activity, synthesis of a collagen matrix,[8] formation of calcium phosphate mineral deposits,[8] and production of bone-related proteins, such as osteopontin, osteonectin, and bone sialoprotein.[9]

Peptides

Design of bioactive and ''control'' peptide sequences

Analysis of known heparin-binding sequences in human vitronectin, in apolipoproteins E and B-100, and in Platelet Factor IV has elucidated putative heparin-binding amino acid sequences of the patterns X-B-B-X-B-X and X-B-B-B-X-X-B-X, where B is a basic amino acid and X is a hydropathic amino acid.[10] In the present study, the amino acid sequences of five bone-related adhesive proteins (specifically, fibronectin, vitronectin, bone sialoprotein, thrombospondin, and osteopontin) from a variety of species (specifically, rat, human, rabbit, mouse, pig, frog, chicken, and cow) were obtained from the SwissProt protein database (accessible on the World Wide Web at http://expasy.hcuge.ch) and examined for the presence of B-B-X-B amino acid patterns, where a basic residue could be either Lysine (Lys), Arginine (Arg), or Histidine (His). It was determined that the B-B-X-B patterns existing in bone-related adhesive proteins most frequently were composed of the amino acids Lysine, Arginine, Serine (Ser), and Arginine, respectively. Therefore, it was hypothesized that the sequence Lys-Arg-Ser-Arg could be a minimal, bioactive, amino acid sequence that promoted proteoglycan-mediated osteoblast adhesion.

In order to experimentally prove the bioactivity of a peptide sequence, an appropriate nonadhesive ''control'' peptide must be utilized in parallel experiments. The control peptide should be similar to the bioactive peptide; for example, studies that utilized the adhesive peptide Arg-Gly-Asp-Ser have used the nonadhesive control peptide Arg-Asp-Gly-Ser.[1,5] One control peptide chosen for use in the present study was Lys-Ser-Ser-Arg since it was composed of the same amino acids (Lysine, Arginine, and Serine) present in the hypothetically bioactive Lys-Arg-Ser-Arg sequence, but did not follow the B-B-X-B heparin-binding pattern.[10] Other sequences such as Lys-Ser-Arg-Arg (which contains the same number of amino acids as the bioactive sequence Lys-Arg-Ser-Arg, but in a scrambled order) were considered as alternative control peptides but rejected since Lys-Ser-Arg-Arg creates a pattern of B-X-B-B, that is, the heparin-binding pattern in reverse.[10] The second control peptide, His-His-Tryptophan (Trp)-His, was chosen for use in the present study because it followed the B-B-X-B pattern but did not contain Lysine, Serine, or Arginine.

Peptide synthesis and analysis

Peptides used in the present study included: the adhesive sequence Arg-Gly-Asp-Ser (RGDS) and the nonadhesive peptide Arg-Asp-Gly-Ser (RDGS); the putative heparin-binding sequences Lys-Arg-Ser-Arg (KRSR), Lys-Arg-Ser-Arg-Gly-Gly-Gly (KRSRGGG), Lys-Arg-Ser-Arg-Gly-Gly-Gly-Gly-Gly-Gly (KRSRGGGGGG), and (Lys-Arg-Ser-Arg)$_4$–Multiple Antigenic Peptide ($KRSR_4$-MAP), where ''Multiple Antigenic Peptide'' is a commercially available tetravalent poly-lysine peptide core; and the nonadhesive, control peptides Lys-Ser-Ser-Arg-Gly-Gly-Gly (KSSRGGG) and His-His-Trp-His (HHWH).

Peptides were synthesized on a BioSearch 9500 automated peptide synthesizer using a Merrifield resin[11] and a tBOC synthesis strategy. Hydrofluoric acid cleavage was performed with the ''low-high'' method,[12] and the peptides were purified using reverse phase chromatography on a Millipore Sep-Pak cartridge followed by HPLC chromatography (C18 column, gradient 0–60% acetonitrile and 0.1% trifluoroacetic acid). Purified peptides were examined by amino acid analysis[13] to confirm composition and concentration and by automated gas phase sequence analysis to confirm amino acid sequences.

Substrate preparation and immobilization of peptides

Peptides were covalently immobilized on borosilicate glass coverslips (transparent, inexpensive, readily available, model substrates) using established techniques,[5,14] techniques that successfully have been used to immobilize approximately 80 p*M* peptide per square centimeter of substrate surface.[5,14] Specifically, glass coverslips were etched for 1 h in a 3:1 (v/v) solution of 2*N* sulfuric acid:2*N* nitric acid, degreased in acetone and ethanol, and rinsed with distilled water. The degreased and acid-etched substrates were immersed in 2% 3-aminopropyltriethoxysilane in dry acetone,[15,16] supplemented with 2% triethylamine,[17] under an Argon environment at 40°C for 1 h. The substrates were rinsed with methylene chloride and acetone and cured under Argon at 120°C for 3 h.[18] At this stage, the silane-coated substrates possessed surface-bound amine groups and thus were classified as ''aminophase'' substrates. Peptides were covalently bound to immobilized amine groups on the substrate surfaces during incubation (under Argon) with a 25:25:1 (v/v/v) solution of 0.1 m*M* peptide in dry N, N-dimethylformamide:1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (2.5 mg/mL in dry N, N-dimethylformamide):N-ethylmorpholine.[19,20] Unreacted amine groups were passivated by soaking peptide-modified substrates in acetic anhydride; adsorbed peptides then were removed with 4*M* urea and 1*M* sodium chloride.[5] Substrates modified with immobilized peptides were rinsed thoroughly in distilled water prior to sterilization and use in experiments with cells.

Substrates that had been aminated but not exposed to peptides, that is, aminophase substrates, were used as control surfaces. Plain glass substrates that had been etched in a 3:1 (v/v) solution of 2*N* sulfuric acid: 2*N* nitric acid, degreased in acetone and ethanol, and rinsed copiously with distilled water also were used as controls. All substrates were sterilized via overnight ultraviolet irradiation prior to use in experiments with cells.

Cell adhesion experiments

Cells (either neonatal rat calvarial osteoblasts, bovine pulmonary artery endothelial cells, or rat skin fibroblasts) were enzymatically lifted from polystyrene tissue culture flasks using small volumes (i.e., less than 1 mL) of low-trypsin EDTA solution (0.05% trypsin, 0.53 m*M* ethylenediaminetetraacetic acid in Ca^{++}-, Mg^{++}-free Hank's buffered saline solution) and suspended in serum-free DMEM.

Cells were seeded onto substrates (specifically, plain glass, aminophase glass, and glass modified with various peptides) at a density of 2,500 cells/cm^2 substrate surface area. The cells then were allowed to adhere for 4 h under standard tissue-culture conditions in serum-free DMEM. At the end of the incubation period, the adherent cells were fixed with 4% formaldehyde in sodium phosphate buffer for 10 min. The fixed cells were stained with Coomassie Brilliant Blue for 10 min and rinsed with distilled water.

The number of adherent cells in each of five random fields per substrate were counted manually using a light microscope (Nikon); the average cell count per substrate was expressed as ''cell density'' or cells/cm^2 of substrate surface area. Data were analyzed using ANOVA techniques and either Duncan's Multiple Range test or appropriate *t* tests;[21] model adequacy checking included residual error analysis and normality checking.[21]

Competitive inhibition of adhesion experiments

Prior to experiments, peptide solutions (specifically, 0.02, 0.2, 2, and 4 m*M*) were prepared in serum-free DMEM and stored at 4°C for not more than 2 weeks. Osteoblasts were enzymatically lifted from tissue culture polystyrene flasks using small (i.e., less than 1 mL) volumes of low-trypsin EDTA solution (0.05% trypsin, 0.53 m*M* ethylenediaminetetraacetic acid in Ca^{++}-, Mg^{++}-free Hank's buffered saline solution), and resuspended (50,000 cells/mL) in serum-free DMEM. Aliquots of this cell suspension then were placed in microcentrifuge tubes with aliquots of a peptide solution, creating final concentrations of 0.01, 0.1, 1, and 2 m*M* peptide in DMEM. Controls were aliquots of the cell suspension placed in microcentrifuge tubes with serum-free DMEM (without peptide). All tubes were maintained at 37°C with gentle mixing for 30 min, after which the tubes were centrifuged at 1,000 rpm for 5 min. All cell pellets then were resuspended in serum-free DMEM and seeded on substrates modified with KRSRGGG. Cells were allowed to adhere on these substrates for 2 h in a standard cell culture environment, after which the adherent cells were fixed with 4% formaldehyde in sodium phosphate buffer for 10 min. The fixed cells were stained with Coomassie Brilliant Blue for 10 min and rinsed with distilled water.

The number of adherent cells in each of five random fields per substrate were counted manually using a light microscope, and the average cell count per substrate was expressed as ''cell density'' or cells/cm^2 of substrate surface area. Data were analyzed using ANOVA techniques and Scheffé's test;[21] model adequacy checking included residual error analysis and normality checking.[21]

RESULTS AND DISCUSSION

Select bioactivity of the KRSR peptide sequence

The novel KRSR sequence exhibited bioactivity that was a function of structural aspects of the peptide, was cell-specific, and proved to be crucial for maximal osteoblast adhesion to substrates. In the present study, osteoblast adhesion in serum-free DMEM was enhanced significantly ($p < 0.05$) on glass modified with the novel adhesive peptide KRSRGGG (Fig. 1) compared to control substrates (plain glass, aminophase glass, glass modified with the nonadhesive peptide RDGS, and glass modified with the nonadhesive peptide KSSRGGG). This bioactivity is unique since HHWH

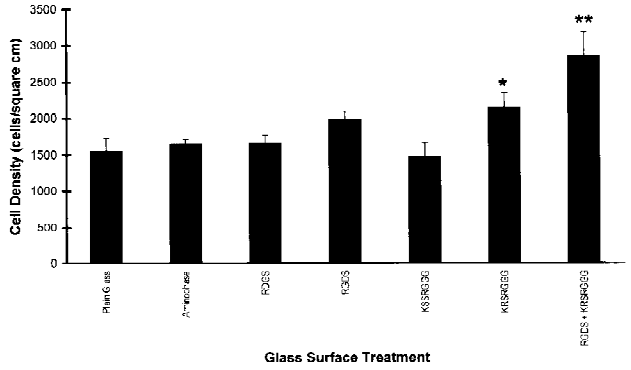

Figure 1. Rat calvarial osteoblast adhesion. The substrates utilized were borosilicate glass coverslips, plain, aminophase, or modified with the following immobilized peptides: RDGS = Arg-Asp-Gly-Ser; RGDS = Arg-Gly-Asp-Ser; KSSRGGG = Lys-Ser-Ser-Arg-Gly-Gly-Gly; KRSRGGG = Lys-Arg-Ser-Arg-Gly-Gly-Gly; RGDS + KRSRGGG = *both* Arg-Gly-Asp-Ser and Lys-Arg-Ser-Arg-Gly-Gly-Gly. Data are mean ± SEM; $n = 8$; *$p < 0.05$; **$p < 0.01$ (Duncan's Multiple Range Test).

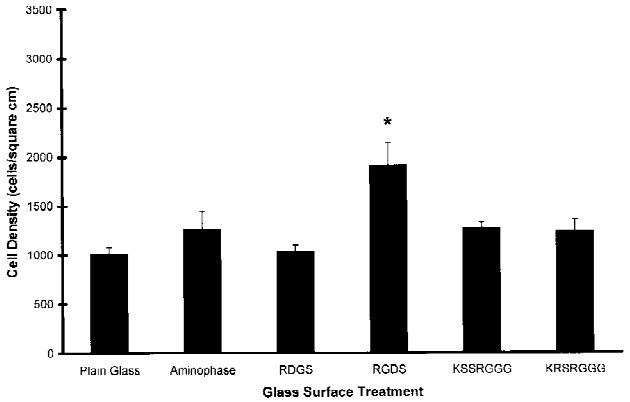

Figure 3. Rat skin fibroblast adhesion. The substrates utilized were borosilicate glass coverslips, plain, aminophase, or modified with the following immobilized peptides: RDGS = Arg-Asp-Gly-Ser; RGDS = Arg-Gly-Asp-Ser; KSSRGGG = Lys-Ser-Ser-Arg-Gly-Gly-Gly; KRSRGGG = Lys-Arg-Ser-Arg-Gly-Gly-Gly. Data are mean ± SEM; $n = 6$; *$p < 0.05$ (Duncan's Multiple Range Test).

(which follows the B-B-X-B pattern proposed by Cardin and Weintraub[10]) did not promote adhesion [specifically, 1,080 ± 85 versus 1,508 ± 135 cells/square cm (mean ± SEM) for substrates modified with HHWH versus KRSR, respectively]. In other words, peptides that follow the B-B-X-B pattern are not universally osteoblast adhesive. Further evidence for the cell specificity was provided by the results that immobilized KRSRGGG peptides selectively promoted adhesion of osteoblasts (Fig. 1) but not of bovine pulmonary artery endothelial cells nor of rat skin fibroblasts (Figs. 2, 3, respectively); therefore, KRSR-containing peptides may be osteoblast- and/or bone-cell specific.

Certain aspects of the KRSR archetype also were investigated to elucidate the role of stereochemistry in the interactions of osteoblasts with these peptide sequences. The adhesion experiments presented in Figure 4 utilized peptides containing ''spacers'' of multiple glycines; the rationale behind this choice was to raise the bioactive KRSR sequence away from the substrate surface and, by allowing flexing and/or rotation of the peptide, possibly to make the KRSR sequence available in a conformation that would be more ''attractive'' to cells. Osteoblast adhesion, however, was similar on substrates modified with KRSR, KRSRGGG, or KRSRGGGGGG peptides (Fig. 4), demonstrating that the spacer lengths utilized in the present study did not mediate/affect osteoblast–proteoglycan interactions with immobilized bioactive peptides. In contrast, osteoblast adhesion in serum-free DMEM on substrates modified with the tetravalent $(KRSR)_4$-MAP was significantly ($p < 0.05$) increased compared to cell adhesion on substrates modified with the KRSR peptide (Fig. 4); these results showed that the mechanism of osteoblast adhesion

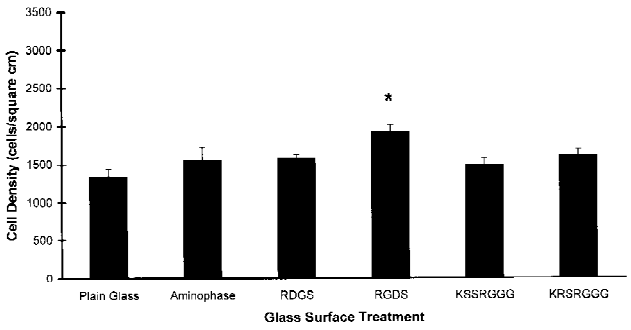

Figure 2. Bovine pulmonary artery endothelial cell adhesion. The substrates utilized were borosilicate glass coverslips, plain, aminophase, or modified with the following immobilized peptides: RDGS = Arg-Asp-Gly-Ser; RGDS = Arg-Gly-Asp-Ser; KSSRGGG = Lys-Ser-Ser-Arg-Gly-Gly-Gly; KRSRGGG = Lys-Arg-Ser-Arg-Gly-Gly-Gly. Data are mean ± SEM; $n = 6$; *$p < 0.05$ (Duncan's Multiple Range Test).

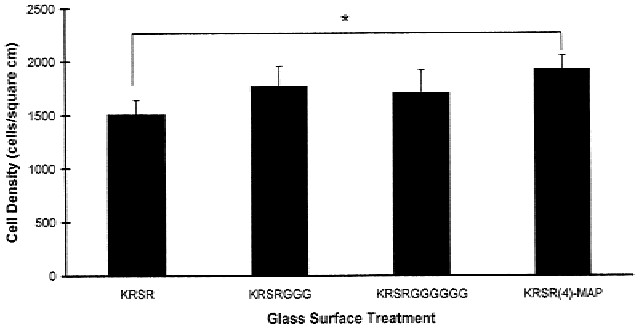

Figure 4. Rat calvarial osteoblast adhesion on substrates modified with various KRSR-containing peptides. The substrates utilized were borosilicate glass coverslips modified with the following immobilized peptides: KRSR = Lys-Arg-Ser-Arg; KRSRGGG = Lys-Arg-Ser-Arg-Gly-Gly-Gly; KRSRGGGGGG = Lys-Arg-Ser-Arg-Gly-Gly-Gly-Gly-Gly-Gly; KRSR(4)-MAP = $(Lys\text{-}Arg\text{-}Ser\text{-}Arg)_4$-Multiple Antigenic Peptide. Data are mean ± SEM; $n = 8$; *$p < 0.05$ (t test).

mediated by KRSR-containing peptides may be sensitive to peptide ''valence'' or density rather than to length.

KRSR-mediated mechanism of osteoblast adhesion

Both integrin- and proteoglycan-mediated mechanisms are crucial for osteoblast adhesion; in the present study (and compared to all substrates tested) osteoblast adhesion was significantly ($p < 0.01$) enhanced on glass modified with *both* the adhesive peptide RGDS and the novel adhesive peptide KRSRGGG (Fig. 1), confirming that part of the osteoblast adhesion mechanism is mediated by RGD-containing peptides.[1,5] It was reported that blocking the heparin-binding sites of Platelet Factor IV (adsorbed on microtiter plates at a concentration of 2.581 mg/mL) with 5,000 units of heparin resulted in a 62% reduction of 1-h adhesion of rat calvarial osteoblasts to the coated plates;[2] this 62% reduction due to the blocking of active heparin sites on the substrate compares very well to the 60.4 ± 5.2% (mean ± SEM) reduction in rat osteoblast adhesion to a heparin-binding substrate (following blockage of cell membrane receptors) observed in the present study (Fig. 5). Synthetic peptides were chosen to investigate osteoblast adhesion in the absence of serum to eliminate/minimize possible nonspecific, random interactions of cell receptors with their ligands. However, since osteoblast adhesion was not completely eliminated (Fig. 5), it is still possible that subsaturation concentrations of heparin-binding agents may have been used in both the present study and in the study of Puleo and Bizios.[2] Alternatively, residual osteoblast adhesion may have been a function of nonproteoglycan-mediated adhesive mechanisms, such as integrin-related mechanisms.

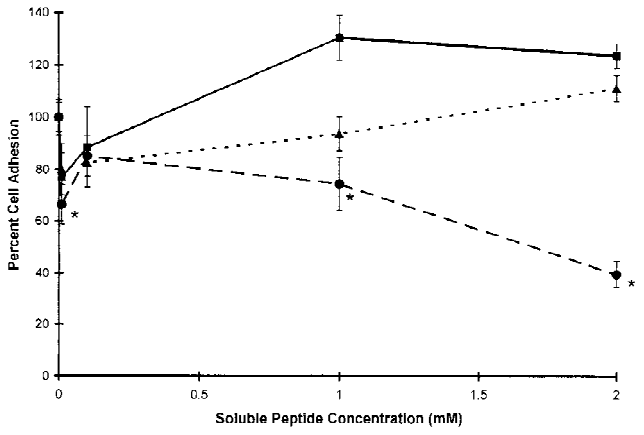

Figure 5. Inhibition of osteoblast adhesion to substrates modified with KRSRGGG. 100% adhesion was defined as the number of osteoblasts, preincubated in serum-free DMEM without peptides, that adhered to substrates modified with KRSRGGG. The various lines in this figure denote preincubation of osteoblasts with the following soluble peptides: —— = KSSRGGG; ---------- = RGDS; – – – – = KRSRGGG. Data are mean ± SEM; $n = 8$; *$p < 0.05$ (Scheffé's test).

The respective adhesion mechanisms are unique, distinct, and important for the function of osteoblasts. Evidence for the uniqueness is provided by the fact that the blocking of osteoblast cell membrane receptors with various concentrations of either soluble KSSRGGG (nonadhesive sequence) or RGDS peptides did not inhibit subsequent, 2-h cell adhesion on substrates modified with immobilized KRSRGGG (Fig. 5). In contrast, preincubation of osteoblasts with KRSRGGG (adhesive sequence) resulted in decreased subsequent cell adhesion on substrates modified with immobilized KRSRGGG peptides (Fig. 5). These data provide evidence that osteoblasts interact with RGDS and KRSRGGG via distinct (i.e., integrin- and proteoglycan-mediated) mechanisms, but they do not completely exclude the possibility of interactions between the KRSRGGG ligand and select integrin receptors.

Integrin receptors (specifically, $\alpha_4\beta_1$ and $\alpha_v\beta_5$) may interact with heparin-binding peptides.[22,23] Both human[24] and rat[25] osteoblastic cells express $\alpha_v\beta_5$ integrin receptors. Human osteoblasts (obtained either from human fracture callous and neonatal costochondral junctions[26] or from osteophytic bone[27]) expressed the β_1 integrin subunit strongly and uniformly[26,27] and the α_4 integrin subunit heterogeneously.[26] Integrin $\alpha_4\beta_1$ was detected only at low levels on human osteoblastic cells.[24] Therefore, while the probability of $\alpha_4\beta_1$ integrin-mediated osteoblast adhesion is low, the possibility of $\alpha_v\beta_5$ integrin-mediated osteoblast adhesion to heparin-binding peptides cannot be ignored.

In an affinity chromatography study, the basic peptides Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg, or RKKRRQRRR, (from the HIV Tat protein) and Lys-Lys-Gln-Arg-Phe-Arg-His-Arg-Asn-Arg-Lys-Gly, or KKQRFRHRNRKG, (from the heparin-binding domain of vitronectin) bound the $\alpha_v\beta_5$ integrin pair from L8 rat skeletal muscle cells and from human SK-LMS leiomyosarcoma cells.[23] The $\alpha_v\beta_5$–Tat peptide interaction was stable in the presence of 10 m*M* EDTA even though integrin–ligand interactions (including the binding of $\alpha_v\beta_5$ to the RGD sequence) typically require divalent cations and thus are inhibited in the presence of the calcium chelator EDTA.[23] Additionally, the $\alpha_v\beta_5$–Tat peptide interaction was not affected by a monoclonal antibody that inhibits RGD-mediated binding of $\alpha_v\beta_5$ to vitronectin.[23] Vogel et al.[23] proposed that $\alpha_v\beta_5$ integrin pairs may possess two distinct binding sites, one for RGD-containing peptides and one for select basic peptides, such as the Tat peptide or the heparin-binding domain of vitronectin. Because $\alpha_v\beta_5$ did not substantially bind to peptides consisting entirely of either eight Arginine or eight Lysine residues, nonbasic residues within primarily basic

peptides may be important for cell–peptide binding specificity.[23] It should be noted that both the Tat peptide and the heparin-binding peptide from vitronectin used by Vogel et al.[23] contain B-B-X-B sequences (specifically, RRQR in Tat and KKQR in vitronectin); thus the results of Vogel et al.[23] are particularly relevant to the present study.

CONCLUSIONS

Considerations for the design of proactive dental/orthopedic implant biomaterials

Because proactive biomaterials must be designed to specifically and selectively encourage appropriate functions of cells and tissues, strategies for the design/selection of bioactive agents (such as peptides) and their use in conjunction with biomaterials and/or implant devices have become important. The present study constitutes an illustrative example of the rational design of bioactive (e.g., adhesive) peptides. First, a hypothesis was formed such that a fundamental biochemical property and/or event (the binding of heparin and heparin-like compounds to proteins) could be studied logically. Second, the literature from a number of fields was reviewed. Third, a computer database (from the wide range of chemical/molecular biology/biochemical databases currently available for use via the World Wide Web) was used to search for select commonalties (e.g., the B-B-X-B sequence) among bone-related adhesive proteins, from a number of species. Finally, the results of the computer search were acted upon decisively.

The present study provides guidelines for the design of proactive dental/orthopedic biomaterials by confirming that osteoblast adhesion to substrates can be controlled selectively and significantly by specific, immobilized, adhesive peptides. Moreover, the present study demonstrates that a proactive biomaterial that enhances only integrin-mediated mechanisms will not maximize osteoblast adhesion. A well-designed proactive biomaterial for dental/orthopedic implants should, therefore, promote both integrin- and proteoglycan-mediated osteoblast adhesion; this goal could be achieved, for example, by modifying the surface of a proactive biomaterial with *both* integrin-binding peptides containing the Arg-Gly-Asp sequence *and* heparan sulfate-binding peptides containing the novel Lys-Arg-Ser-Arg sequence.

While osteoblast adhesion certainly is a crucial process at the bone–implant interface, biomaterial surfaces modified with adhesive peptides alone will not optimize conditions for multiple osteoblast functions (such as proliferation, deposition of mineralized matrix, etc.) that occur after cell adhesion and that are necessary for the success of an implant.[14] Proactive dental/orthopedic biomaterials should be designed to control multiple osteoblast functions, for example, by modifying the surface of proactive implants with covalently-bound, select adhesive peptides and by carefully mediating the spatial distribution, time course, and type(s) of growth factors and cytokines at/near the bone–implant interface. As the disciplines of cell and tissue engineering develop and mature, future cellular- and molecular-level research will expand and refine the criteria and strategies for proactive biomaterial design that have been elucidated to date.

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