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Adhesion of MC3T3-E1 cells bound to dentin phosphoprotein specifically bound to collagen type I

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Abstract

Dentin sialophosphoprotein (DSPP) is a member of the SIBLING (small integrin binding N-linked glycoprotein) family of proteins commonly found in mineralized tissues. Dentin phosphoprotein (DPP) is a naturally occurring subdomain of DSPP that contains the cell binding RGD sequence. Previously, the orientation and conformation of other SIBLING family members specifically bound to collagen I have been investigated with respect to their cell adhesion properties. In this study, the orientation of DPP under similar circumstances is examined, and the results are discussed relative to the previous investigations. Radiolabeled adsorption isotherms were developed for DPP adsorbing to both tissue culture polystyrene (TCPS) and collagen coated TCPS. Then, a MC3T3-E1 cell adhesion assay was performed on TCPS and collagen coated TCPS in the presence of identical amounts of adsorbed DPP. It was discovered that there was a significant difference in the number of bound cells on the TCPS and collagen coated TCPS, with a preference for TCPS. Furthermore, a cell inhibition assay was conducted to confirm that the cell binding that occurred was due to specific integrin interactions with the RGD sequence of DPP. These results suggest that the orientation of DPP, rather than its conformation, dictates the accessibility of the cell binding RGD domains of DPP and that the RGD sequence in DPP is less accessible when DPP is specifically bound to collagen. The results obtained in this study are in stark contrast to previous studies with related SIBLING proteins, and suggest that DPP does not play a key role in cell binding to the collagen matrix of developing bone.

Keywords

dentin phosphoprotein; collagen I; orientation; conformation; MC3T3-E1 adhesion

INTRODUCTION

The extracellular matrix (ECM) is a highly complex mixture of proteins and growth factors, which regulate cell behavior and provide structural support to cells. One of the primary structural proteins in the ECM of the body is collagen. Collagen provides mechanical strength, stability, and an organizational framework in a tissue dependent manner. In bone tissue, for example, the ECM is comprised of 10–30 mass% protein, with the remaining 70–90 mass% being hydroxyapatite (HA).[1] Roughly, 90% of the protein content of the ECM has been shown to be collagen, primarily type I.[1,2] In the initial stages of bone formation,

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cells lay down a matrix of collagen. After forming this network, the cells excrete non-collagenous proteins into the matrix and the matrix is then mineralized to form bone.[2] While several proteins have been identified throughout bone tissue, the primary group of noncollagenous proteins is the SIBLING (small integrin binding N-linked glycoprotein) family, which is composed of five members including osteopontin (OPN), bone sialoprotein (BSP), dentin sialophosphoprotein (DSPP), matrix extracellular glycoprotein, and dentin matrix protein 1.[1–6]

For improved wound healing to occur via implanted biomaterials, the surface of the biomaterial must be able to control the adsorption, orientation, and conformation of proteins that adsorb immediately upon implantation. It has been suggested that the foreign body response is activated by this nonspecific adsorption process and that it may be reduced though material design.[7] The initial efforts to control protein orientation and conformation upon adsorption were focused on using charged surfaces that could orient a protein based on its natural dipole distribution. These studies began with biosensor applications in an effort to improve the sensor sensitivity.[8–11] Charged surfaces were then adapted to control the bioactivity of adsorbed proteins for biomaterials applications.[12] For example, a study by Liu et al. demonstrated that OPN had a preferential orientation for endothelial cell adhesion, when adsorbed to a positively charged —NH_2 terminated self-assembled monolayer (SAM) as opposed to a negatively charged —COOH terminated SAM.[13] These investigations were taken further in a study that showed that OPN, oriented by underlying surface charge, could reduce the foreign body response to an implanted polymeric hydrogel.[14]

A second approach for controlling the orientation and conformation of adsorbed proteins is to recapitulate protein–protein and protein–substrate binding interactions that are highly specific and naturally occurring. This approach has been adapted for both biosensor and biomaterial applications.[15–19] For example, our previous study has examined MC3T3-E1 osteoblast-like cell adhesion onto surfaces covered with either OPN or BSP when the proteins were naturally oriented through their specific protein–protein binding interactions with collagen or randomly adsorbed onto tissue culture polystyrene (TCPS) substrates.[20,21] A statistically significant difference was detected in the amount of cell binding on the collagen–OPN surfaces, indicating a preferential orientation and/or conformation for MC3T3-E1 cell adhesion when OPN was specifically bound to collagen.[20] However, no statistically significant differences were found in the MC3T3-E1 cell adhesion when comparing BSP specifically bound to collagen versus randomly adsorbed on TCPS. This suggests that the accessibility of the arginine–glycine–aspartic acid (RGD) amino acid sequence is mediated by the conformational flexibility of BSP.[20] In this investigation, the naturally occurring subdomain of DSPP containing the RGD cell binding sequence, dentin phosphoprotein (DPP), is studied to better formalize the role that this protein plays aiding cellular adhesion to the ECM of developing bone.

DSPP was originally thought to occur only in dentin, but has since been found in bone tissue as well.[1,22] DPP is a cleavage product of DSPP, the other cleavage product being dentin sialoprotein (DSP).[23] DPP is highly negatively charged throughout the molecule and it is comprised of 751 amino acids in humans, with a molecular weight of 100–140 kDa.[1,6] It is the major noncollagenous protein of the dentin ECM.[23] Additionally, DPP contains a large number of aspartic acid–serine–serine (DSS) repeats, which are believed to play a key role in Ca^{2+} sequestration and the subsequent HA mineralization of bone and dentin.[1,24,25] The DPP subdomain also contains an RGD cell binding motif, which encourages cell binding by interacting with transmembrane integrins on cells.[26] DPP has also shown a strong affinity for collagen binding.[1,2,6,24,27] Because DPP contains both an RGD cell binding moiety and it has been localized ahead of the mineralized front of bone and dentin, it is possible that it

plays a role in mediating cell binding in these developing tissues.[22] This would be in addition to its possible roles in bone and dentin mineral formation and growth.

The foci of this work are to (a) probe the natural orientation of DPP with respect to its cell binding domain, when it is specifically bound to collagen or randomly adsorbed to TCPS and (b) compare the overall cell binding capabilities of DPP to previous study with OPN and BSP.[20,21] This study provides valuable insight into the relative importance of DPP for promoting cellular adhesion to the collagen matrix of developing bone, especially relative to OPN and BSP. While performing this study, it was important to ensure that there were identical amounts of DPP bound to both the TCPS and collagen coated substrates. This was accomplished by developing ^{125}I radiolabeled adsorption isotherms that were subsequently used to determine DPP exposure concentrations that result in equal amounts of adsorbed protein on the two substrates. Cell adhesion assays were conducted with MC3T3-E1 osteoblast-like cells and inhibition assays were performed with a GRGDSP peptide to confirm that the cell adhesion was due to integrin interactions with the RGD sequence of DPP. In this study, it was found that DPP does not have a favorable orientation for promoting cell binding when specifically bound to collagen. This suggests that DPP does not play a major role in promoting cellular adhesion to the matrix of developing bone, especially relative to OPN and BSP.

MATERIALS AND METHODS

Materials

Ultrapure water (18.2 MΩ-cm) was obtained from a Millipore Synergy UV water purification system (Billerica, MA) and used for all experiments. TCPS flasks were purchased from Corning (Corning, NY). To obtain TCPS substrates, sterile flasks were scored into 5 mm × 5 mm with a drill press and broken apart just prior to use in experiments. NaCl and tris(hydroxymethyl)aminomethane (Tris–HCl) were purchased from Thermo-Fisher Scientific (Waltham, MA). NaCl–Tris buffer was prepared by dissolving 25 mM Tris–HCl and 125 mM NaCl in 18.2 MΩ-cm water and adjusting the pH to 7.4 with 1.0 *M* NaOH (Sigma-Aldrich, Saint Louis, MO). Type I collagen from rat tail with a purity of >90% was purchased from BD Biosciences (Bedford, MA). Bovine serum albumin (BSA) with a purity of >96% was purchased from Sigma-Aldrich. Heat denatured BSA was prepared by dissolving 1 mg/mL of BSA in NaCl–Tris buffer and heating at 60°C for 30 min. A 4% paraformaldehyde solution was made by dissolving paraformaldehyde (Thermo-Fisher Scientific) in pH 7.4 phosphate buffered saline (PBS, Sigma-Aldrich) at 60°C until the solution became clear. Hematoxylin was purchased from Acros Organics (Geel, Belgium). A soluble GRGDSP peptide was purchased from Calbiochem (La Jolla, CA). All other cell culture supplies, including fetal bovine serum, α-minimum essential medium (α-MEM), penicillin–streptomycin, trypsin–ethylenediaminetetraacetic acid (trypsin–EDTA, 0.05%, 0.53 m*M*), and soybean trypsin inhibitor were purchased from Invitrogen (Carlsbad, CA). All buffer solutions and cell culture media were filter sterilized with 0.22 μm vacuum filters and stored at 4°C prior to use. MC3T3-E1 osteoblast-like cells (subclone 14, ATCC# CRL-2594) were obtained from ATCC (Manassas, VA).

Substrate preparation

Collagen-TCPS and TCPS substrates were prepared using previously established procedures.[20,21] Briefly, TCPS squares were incubated in 1 mL of NaCl–Tris buffer overnight at room temperature. Afterward, they were rinsed extensively with 18.2 MΩ-cm water and dried with filtered air before being used immediately in subsequent experiments. Collagen–TCPS substrates were prepared by soaking TCPS squares in 1 mL of a 50-μg/mL collagen solution in NaCl–Tris buffer overnight at room temperature. Following this, the

substrates were removed from the collagen solution, rinsed extensively with 18.2 MΩ-cm water, dried with filtered air, and then soaked in 1 mL of 1 mg/mL heat denatured BSA for 5 h to block nonspecific protein binding to any exposed TCPS. Afterward, the TCPS–collagen substrates were rinsed with 18.2 MΩ-cm water, dried with filtered air, and then used immediately in subsequent experiments.

Dentin phosphoprotein isolation

The noncollagenous proteins including DPP were extracted and isolated from the incisor dentin of 10-week-old rats by standard procedures as described earlier.[28,29] For the separation of noncollagenous proteins including DPP, the rat dentin extracts were first passed through a Sephacryl S-200 (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) gel chromatography column.[29] The Sephacryl S-200 column separated noncollagenous proteins into four major fractions, and an earlier fraction known as ES1 contained a group of higher molecular weight proteins, which included DPP. The ES1 fraction (containing DPP) was loaded onto a Q-Sepharose (Amersham Biosciences, Piscataway, NJ) ion-exchange column connected to a fast-protein liquid chromatography system, and it was eluted within a gradient ranging from 0.1 to 0.8 *M* NaCl in 6 *M* urea (pH 7.4). Then, the fractions enriched with DPP were passed through a Bio-Gel A50m size exclusion column (Bio-Rad, Hercules, CA). The fractions from the Bio-Gel A50m column that contained the most highly pure DPP were combined, dialyzed against water, lyophilized, and used for this study.

Protein adsorption isotherms

DPP was labeled with ^{125}I using iodogen reagent and a modified version of a previously published procedure.[30] Briefly, 100 μg of iodogen was suspended in 35 μL of a 1.5-mg/mL DPP solution in ultrapure water, to which 750 μCi of ^{125}I–Na (100 mCi/mL) was added. After 5 min, the mixture was transferred to a 20-cm Sephadex G25-150 column that had been equilibrated with PBS (pH 7.4). The column was then eluted with 15 mL of PBS and 500 μL fractions were collected. The radioactivity associated with each fraction was determined, and the highest count radiolabeled fraction was selected and used in all adsorption isotherm experiments.

^{125}I radiolabeled DPP was added to 1.0 mg/mL solutions of unlabeled DPP to obtain solutions with a specific activity of 116.0 counts per minute (CPM) per nanogram of protein. TCPS and collagen coated TCPS were prepared as described above. Following the buffer soak (TCPS) or heat denatured BSA soak (TCPS–collagen), the substrates were extensively rinsed with 18.2 MΩ-cm water and then incubated with 1, 10, 50, and 100 μg/mL DPP overnight at 4°C in a humidified atmosphere. Afterward, they were rinsed three times with NaCl–Tris buffer to remove loosely bound proteins. The CPM radioactivity of all of the samples was measured with a Wizard 1470 automatic gamma counter (PerkinElmer, Waltham, MA). The amount of protein specifically adsorbed to the surface of the substrates was calculated by relating the CPM of each sample to the sample surface area and specific activity of the original DPP protein mixture. Each exposure concentration and substrate combination was repeated three times and the data are presented as the mean ± standard deviation of these trials.

Cell culture

MC3T3-E1 cells were continuously grown on TCPS flasks in α-MEM, which was supplemented with a 1% penicillin–streptomycin solution and 10% fetal bovine serum in a humidified atmosphere at 37°C and 5% CO_2. To passage, the cells were rinsed twice with 10 mL of NaCl–Tris buffer followed by incubation in 2 mL of trypsin–EDTA. After the cells detached from the flask, they were resuspended in supplemented α-MEM and replated

onto new TCPS flasks. The cells were passaged once per week and passages 5–10 were used for experiments.

Cell adhesion assay

The cell adhesion assay is similar to that used previously to examine the orientation of proteins specifically bound to collagen.[20,21] TCPS and collagen coated TCPS squares were prepared as described earlier. The protein adsorption steps were identical to those performed in the adsorption isotherm experiment, with the exception that only one concentration was used for each type of substrate and only native, unlabeled protein was used. Specifically, TCPS substrates were exposed to 32.5 μg/mL of DPP while TCPS–collagen substrates were exposed to 50 μg/mL of DPP. The substrates were incubated overnight in a humidified atmosphere at 4°C. Following the overnight adsorption, substrates were placed in a 24-well culture plate where they were rinsed three times with 1 mL of NaCl–Tris buffer and then blocked with 1 mL of 1 mg/mL heat denatured BSA for 30 min. In the meantime, freshly confluent MC3T3-E1 cells were detached with 2 mL of trypsin–EDTA and resuspended in 5 mL of 5 mg/mL soybean trypsin inhibitor in PBS at pH 7.4. The cells were then centrifuged for 5 min at 1000 rpm, after which the supernatant was removed and the cells were washed twice with 10 mL of 5 mg/mL BSA in serum-free α-MEM. Following this, the cells were resuspended in serum-free α-MEM and diluted to a final concentration of 1×10^5 cells/mL, as determined with a hemocytometer. The cells were incubated for 15 min in α-MEM before use in the cell adhesion assay. After the BSA blocking of the well plates was complete, the BSA was removed from the wells and the samples were rinsed three times with 1 mL of NaCl–Tris buffer. Following the rinsing step, 1 mL of cell solution was added to each well and the well plates were incubated for 2 h in a humidified atmosphere with 5% CO_2 at 37°C. Three samples of each substrate type were prepared for each assay and the assay was performed three times.

Cell inhibition assay

The cell inhibition assay was performed in a similar fashion to the cell adhesion assay, with one exception. Before the addition of the dilute cells to the samples, the cells were first incubated with 1 m*M* of a soluble GRGDSP peptide in α-MEM for 15 min.[31] This incubation step replaced the final cell incubation step in the adhesion assay procedures. Three substrates were prepared for each assay, and the assay was performed three times.

Cell fixation and staining

After the cell adhesion and inhibition assays, the cell solution was removed from the wells and the wells were washed three times with warm (37°C) NaCl–Tris buffer to remove loosely bound cells. After this, the cells were fixed by adding 1 mL of 4% paraformaldehyde solution to each well for 5 min. The samples were then rinsed three times with 1 mL of warm NaCl–Tris buffer and then stained with 1 mL of hematoxylin for 5 min. Next, the samples were rinsed extensively with ultrapure water and exposed to 1 mL of warm NaCl–Tris buffer for 3 min. The samples were then rinsed three times with ultrapure water and dried in air. Three 10X brightfield images from each sample were randomly selected and captured using a Nikon Eclipse Ti optical microscope (Shinjuku, Tokyo, Japan) equipped with a Nikon DS-2MBW camera and NIS Elements—BR 3.1 software (Nikon).

Data analysis

The number of adherent cells was used to compare the response of the MC3T3-E1 cells to the two substrate–DPP combinations. The total number of cells that adhered to each sample were physically counted using NIS Elements—BR 3.1 software from each of the images that were captured. A total of 27 images from nine independently prepared samples were

analyzed for each protein–substrate combination. The sample data are presented as the average of all of the images obtained and the error bars represent the standard error of the mean. Sample results were analyzed using one-way analysis of variance and they were considered statistically significant when they had a probability value less than 0.05 ($p < 0.05$). Statistical analysis was performed using OriginPro 8.5 (OriginLab Corporation, MA).

RESULTS AND DISCUSSION

The high purity of the DPP used in this study was confirmed with polyacrylamide gel electrophoresis (SDS-PAGE) using 5 ± 15% gradient gels and Stains-All staining.[29] The results shown in Figure 1 show that the rat dentin DPP used in this study migrated between the 83 and 115 kDa molecular weight markers. This is consistent with the migration rate of rat DPP as reported by Butler et al.[32]

To properly compare the orientation and/or conformation of DPP specifically bound to collagen with respect to its cell binding capabilities, it is important to have identical amounts of protein adsorbed to all of the substrates under comparison. This was accomplished by developing ^{125}I radiolabeled adsorption isotherms for DPP on collagen–TCPS and untreated TCPS. The adsorbed amount of protein was calculated using the specific measured radioactivity of each protein solution before adsorption and the activity of the TCPS or collagen–TCPS substrate after protein adsorption and extensive rinsing. The resulting adsorption isotherms are shown in Figure 2 and confirm that DPP was adsorbed to the TCPS substrates and specifically bound to collagen on the collagen–TCPS substrates. In this figure, it can be observed that DPP exhibited a higher affinity for untreated TCPS at exposure concentrations of 50 and 100 μg/mL, while more protein was absorbed on the collagen–TCPS at concentrations of 1 and 10 μg/mL DPP. It is expected that DPP would adsorb more readily to the TCPS substrate, especially at higher concentrations, because of differences in the number of binding domains available on the two substrates. This trend was similar to that seen in previous related studies with OPN and BSP.[20,21] It should be noted that the total amount of adsorbed protein is higher in this study than corresponding studies with OPN and BSP, confirming that DPP has a strong affinity for collagen.[20,21,24] The isotherms were used to determine concentrations that would result in identical amounts of adsorbed protein on both substrates. Specifically, an exposure concentration of 50 μg/mL DPP was used on the collagen–TCPS substrate and 32.5 μg/mL DPP was used on the TCPS control in subsequent cell binding experiments. The presence of identical amounts of protein on each of the substrates allows for direct comparisons of the two substrates tested without the need for data normalization.

After establishing DPP concentrations that resulted in identical amounts of adsorbed protein on both substrates, cell binding assays were performed to probe the orientation and/or conformation of DPP with respect to its cell binding domain, when specifically bound to collagen type I. Because there are no specific binding interactions between DPP and TCPS, this substrate demonstrates the accessibility of the cell binding sequence when DPP has a random orientation or conformation. At the same time, DPP is known to have a specific binding interaction with collagen. Therefore, this substrate should demonstrate the native orientation or conformation of DPP when specifically bound to collagen. Similar studies have been performed for both OPN and BSP.[20,21] In these previous studies, it was found that OPN has a positive orientation for cell binding when specifically bound to collagen while the cell binding properties of BSP appear to be mediated by the conformational flexibility of the protein.[20]

Figure 3(a, b) shows representative light microscopy images of the cell binding to both TCPS and collagen–TCPS in the presence of DPP. In these images, it can be seen that there

is a slight preference for cell binding to the TCPS substrate when compared with the collagen–TCPS substrate. This suggests that the specific binding interactions between collagen and DPP lead to a negative or unfavorable orientation and/or conformation of DPP for cell binding. Figure 3(c, d) shows representative light microscopy images from the cell inhibition assay for both substrates in the presence of DPP. The fact that all cell binding is essentially eliminated when the cells are first exposed to the GRGDSP peptide confirms that the cell binding seen in Figure 3(a, b) is through cell integrin interactions with the RGD sequence of DPP. To confirm that the differences in the cell binding are not caused by the different underlying substrates, control studies were conducted with heat denatured BSA. Representative light microscopy images for these controls can be seen in Figure 4(a, b). These images confirm that the differences in the level of bound cells seen in Figure 3(a, b) are due to differences in the accessibility of the RGD sequence in DPP and not the underlying substrate composition. This is further supported by the quantitative analysis completed over multiple images and independently prepared samples. These results are shown in Figure 5. In this figure, it can be seen that there is a two- to three-fold increase in the number of adherent cells in the presence of DPP when compared with the BSA controls. In addition, there are ~ 40% more cells bound to the TCPS control when compared with the collagen–TCPS substrate in the presence of DPP. The difference between the DPP substrates was also determined to be statistically significant ($p = 0.015$).

These results demonstrate that the RGD cell binding sequence in DPP is less accessible when the protein is specifically bound to collagen. Further insight into the role of conformation versus orientation can be gained by comparing the results of this study to those obtained earlier with both OPN and BSP.[20] OPN was found to have a favorable orientation for cell binding because it had an intermediate amount of bound cells when randomly oriented on TCPS and a nearly confluent coverage of cells when bound to collagen–TCPS. Alternatively, the cell binding properties of BSP were found to be dictated by its conformational flexibility because there was nearly confluent coverage of cells on both substrates. If the cell binding properties of DPP were dictated by conformational flexibility, it would be expected that a nearly confluent layer of bound cells would be obtained with the TCPS control substrate. Rather, an intermediate surface coverage was found, similar to OPN. In addition, the cell adhesion results obtained with BSP had no statistically significant difference between the two substrates, indicating an equal accessibility to the RGD sequence. This was not the case with OPN or DPP. While the binding interaction between OPN and collagen lead to a three-fold increase in the number of adherent cells suggesting a positive orientation for cell binding, the amount of adherent cells in the presence of DPP was reduced when the protein was bound to collagen, suggesting a negative orientation for cell binding. The results obtained in this study, when analyzed in combination with related previous work, indicate that there is an orientation dependence of DPP with regard to its cell binding properties.

An important feature of DPP is the large number of DSS repeats throughout its amino acid sequence. These DSS repeats are highly negative and are believed to play a role in Ca^{2+} chelation and subsequent mineral formation. In addition, DPP has been shown to adopt a more sheet-like structure once calcium has been bound to these DSS sequences.[1] Given these properties, it is possible that DPP plays a role in cell binding to the mineral matrix of developing bone rather than the collagen matrix. Our previous work demonstrated that OPN has a negative orientation for cell binding to a HA matrix.[16] When combined with the results obtained in this study, it is possible that DPP could play an opposite role to OPN and this study is currently under investigation.

CONCLUSION

In this study, DPP adsorption isotherms were obtained on both TCPS and collagen coated TCPS substrates by radiolabeling. These isotherms were then used to identify conditions that lead to identical amounts of adsorbed proteins for use in subsequent MC3T3-E1 cellular adhesion assays to gain insight into the conformation and/or orientation imparted to DPP based on its specific binding interactions with collagen. It was shown that there were significantly lower levels of cell adhesion when DPP was specifically bound to collagen I as compared with the TCPS control. There were also noticeably fewer adherent cells in the presence of DPP as compared with previous studies with OPN and BSP, even though there were greater amounts of protein present.[20,21] This suggests that DPP does not play a role in cellular adhesion to the collagen matrix of developing bone.

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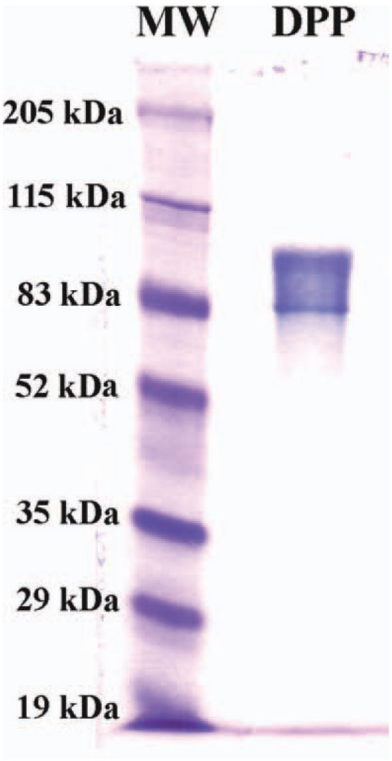

FIGURE 1.
SDS-PAGE and Stains-All staining of DPP isolated from rat dentin incisors. Two micrograms of DPP were loaded onto 5–15% gradient gel. The gel was stained with Stains-All. Note the high purity of DPP. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

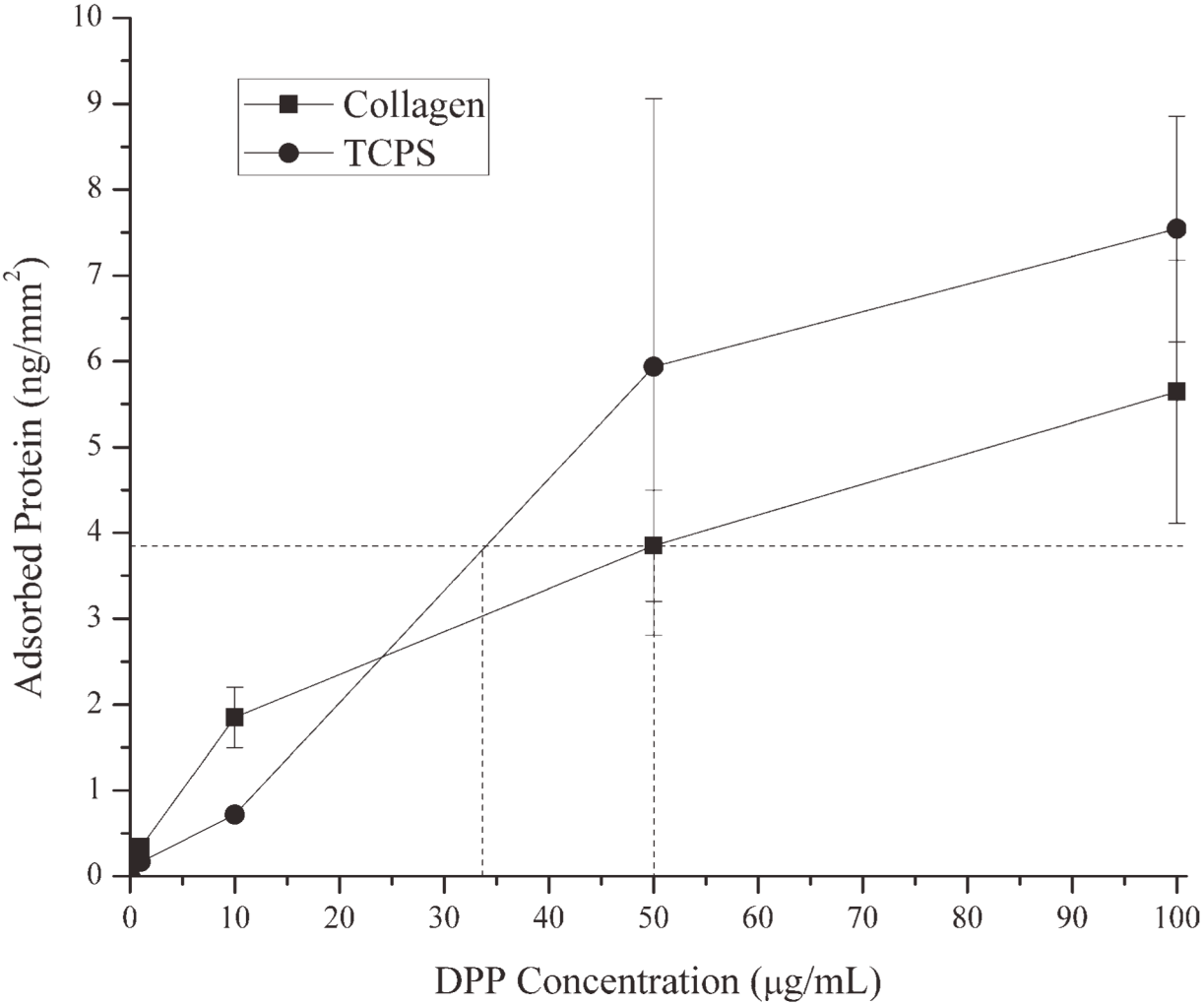

FIGURE 2.

^{125}I radiolabeled adsorption isotherms for DPP on TCPS (circles) and collagen coated TCPS (squares). The dotted lines represent the concentrations used in the cell adhesion and inhibition assays. The data are shown as the mean ± standard deviation (n = 3).

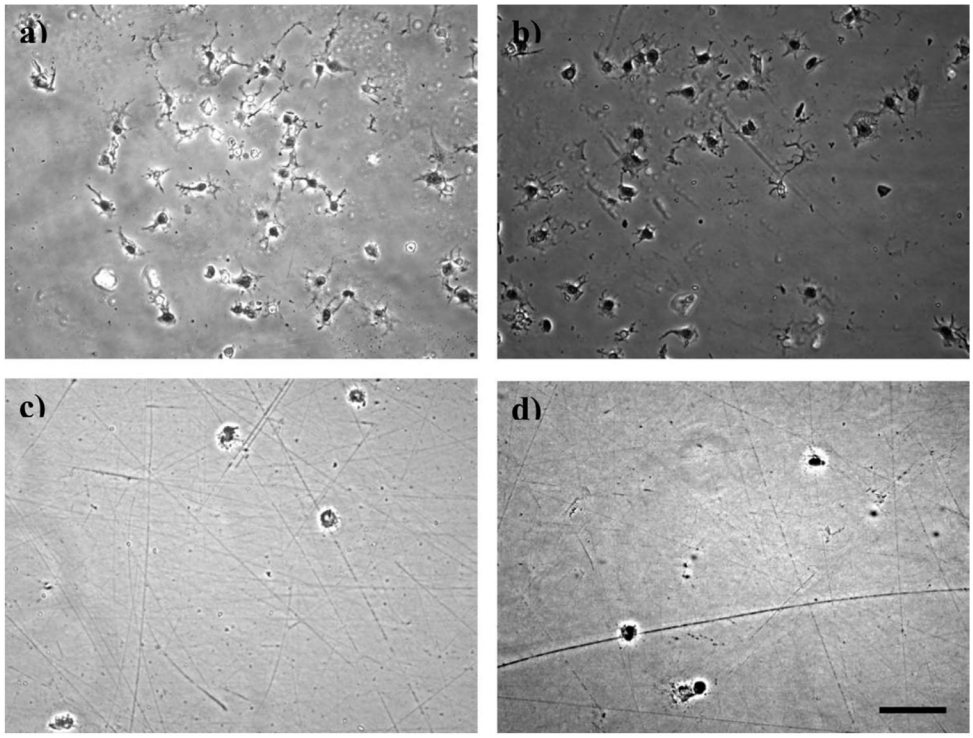

FIGURE 3.

Optical microscopy images of MC3T3-E1 cell adhesion on different substrates: (a) 32.5 μg/mL DPP adsorbed on TCPS; (b) 50 μg/mL DPP adsorbed to collagen coated TCPS; (c) 32.5 μg/mL DPP adsorbed on TCPS in the presence of 1.0 mM GRGDSP; and (d) 50 μg/mL DPP adsorbed to collagen coated TCPS in the presence of 1.0 mM GRGDSP. The scale bar represents 100 μm.

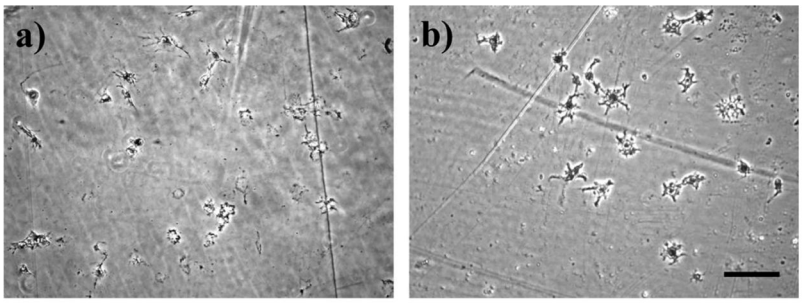

FIGURE 4.

Optical microscopy images of MC3T3-E1 cell adhesion to the control substrates: (a) 1 mg/mL heat denatured BSA adsorbed to TCPS and (b) 1 mg/mL heat denatured BSA adsorbed to collagen coated TCPS. The scale bar represents 100 μm.

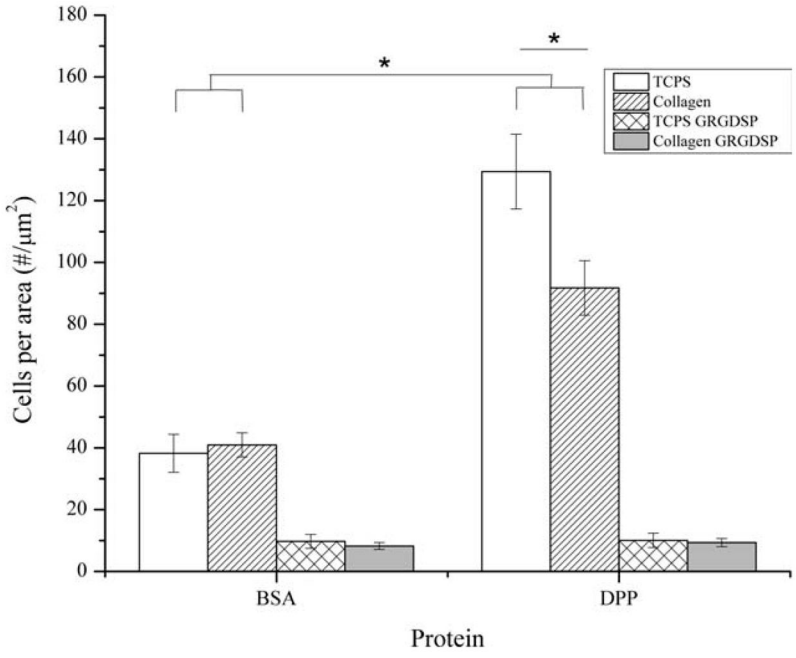

FIGURE 5.

Average number of MC3T3-E1 cells (cells/mm^2) that adhered to TCPS and collagen coated TCPS with adsorbed BSA or DPP in the presence or absence of 1.0 m*M* GRGDSP. The adhesion and inhibition data are presented as the mean ± standard error of the mean from nine samples completed over a total of three separate occasions. Three optical microscopy images were collected and analyzed for each sample completed ($n = 27$). *Represents a statistically significant difference between the surfaces being compared ($p < 0.05$).