

Supporting information

General: The PRGDGGGGGY peptide was purchased from Anygen Corp. (Korea). The purity of the peptide was 95.9 % as certificated by high performance liquid chromatography (HPLC) and Mass spectrometry. Tyrosinase from mushroom (lyophilized powder and ≥ 1000 units/mg solid), tyramine (TA), dopamine (DA), and fluorescein isothiocyanate (FITC) were supplied from Sigma–Aldrich. TiO₂ substrates (10 mm × 10 mm, and thickness = 1.0 mm) were obtained from Seoul titanium Inc. (South Korea). All chemicals were used without further purification. For *in vitro* cell culture, α -minimal essential medium (α -MEM), fetal bovine serum (FBS), trypsin/EDTA, penicillin-streptomycin (PS), and phosphate buffered saline (PBS, pH 7.4) solution were purchased from Gipro BRL (Carlsbad, CA, USA). Rhodamine-phalloidin (Molecular Probes, Eugene, OR) and Hoechst 33258 (Molecular Probes, Eugene, OR) were supplied from Invitrogen Corp.

***In situ* conversion ratio of tyramine (TA) to dopaquinone via tyrosinase-catalyzed reaction:** To determine molecular conversion of phenol molecules to *o*-quinone, the oxidation rate of phenol groups in TA molecules through the tyrosinase-catalyzed reaction was monitored by ultraviolet–visible (UV–Vis) spectroscopy. 1 mL of the TA solution (10 mg/mL dissolved in 0.01 M PBS) was prepared in an UV viable cubic cell and then 20 μ L of tyrosinase (10 KU/mL dissolved in 0.01 M PBS) was added to the cell. The molecular conversion was measured by UV spectroscopy at a wavelength of 275 nm for 30 min. Fig. S1 shows UV spectra of molecular conversion via the tyrosinase-catalyzed oxidative reaction. In the figure, the number indicates the reaction time after treatment with tyrosinase (eg. T1 indicates an absorbance value of the molecule after 1 min). As shown in Fig.

S1A and S1B, the absorbance value at 275 nm, which corresponds to TA, decreased within a few seconds. When higher concentrations of tyrosinase were used, the peak intensity dramatically decreased due to an acceleration in the oxidative reaction at the higher tyrosinase concentrations. This result suggests that the reaction rate could be controlled by varying the tyrosinase concentration. For examples, 30% of the TA molecules treated with 0.2 KU/mL tyrosinase were converted within five minute, while 70% of the molecules treated with 0.4 KU/mL tyrosinase were converted over the same time period. These results demonstrated that the oxidative reaction proceeded rapidly within five minutes and the reaction rate could be easily controlled by changing the tyrosinase concentration. Furthermore, the tyrosinase-catalyzed reaction was a rapid and simple way to convert phenol groups in TA molecules to *o*-quinone, which can be anchored to the substrates in a short time.

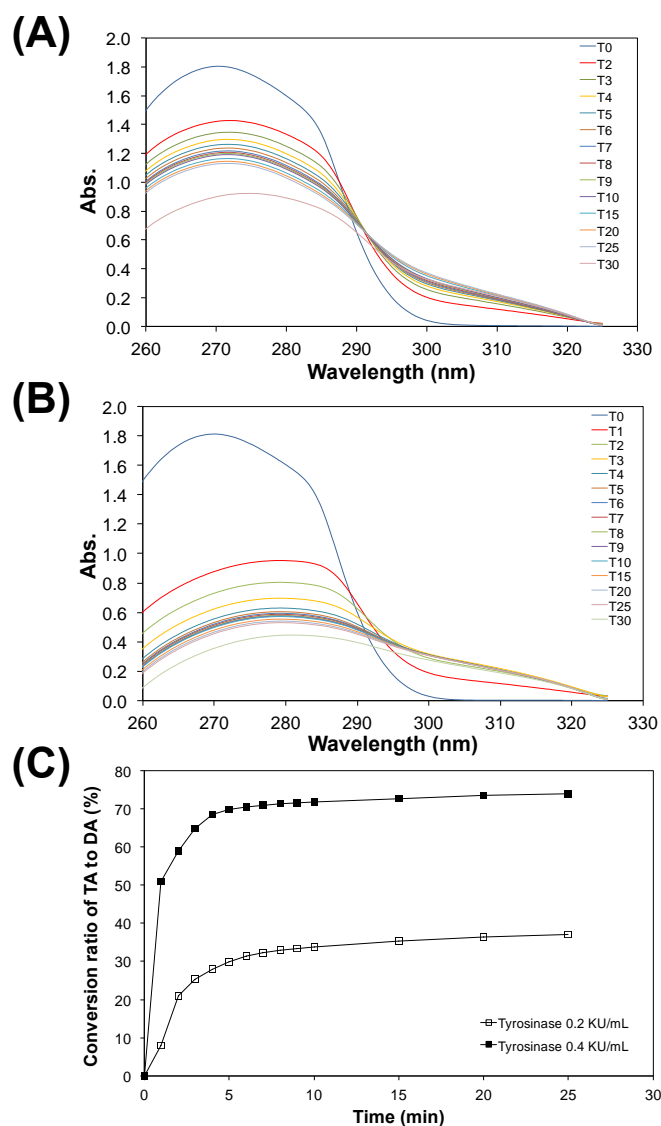


Fig. S1. UV spectra of TA molecules after treatment with tyrosinase as a function of function and at different tyrosinase concentrations: (A) 0.2 KU/mL and (B) 0.4 KU/mL. The molecular conversion ratio of Tyr to dopaquinone via the tyrosinase-catalyzed reaction at different concentrations of tyrosinase (C).

Preparation of PRGDGGGGY-immobilized TiO₂ substrates via tyrosinase-catalyzed reaction: Before the immobilization of the peptide, the TiO₂ substrates were washed by sonication in an ethanol and acetone solution for 15 min. After washing process, the 100 µg/mL of the PRGDGGGGY(RGD-Y) peptide was

dissolved in a 0.01 M of phosphate buffered saline (PBS) buffer solution, and TiO_2 substrates were immersed into the peptide solution. And then, the tyrosinase solution was added into the polymer solution, and the immobilization reaction was carried out for 10 min. After 10 min, the peptide-immobilized substrates were taken out, and washed with distilled water, and dried in a vacuum oven. Although the immobilization reaction was saturated within 1 h, the samples prepared by conjugating the peptide onto the TiO_2 substrates for 10 min were used for this study to emphasize the fast immobilization rate of our system, which have effective cell adhesive properties on the substrates. Fig. S2 shows a schematic of the procedure used for RGD–Y immobilization onto TiO_2 substrates via the tyrosinase–catalyzed reaction. The RGD–Y conjugated TiO_2 substrates were characterized by measuring static water contact angles using the surface electro optics (SEO phoenix 150, South Korea) (Fig. S3) and X-ray photoelectron spectroscopy (XPS) using K–Alpha (Thermo electron, U. K.).

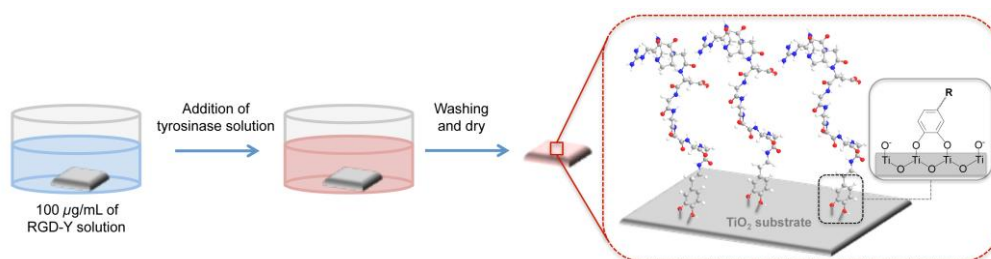


Fig. S2 Schematic representation of the procedure used for RGD–Y immobilization onto TiO_2 substrates via a tyrosinase–catalyzed reaction.

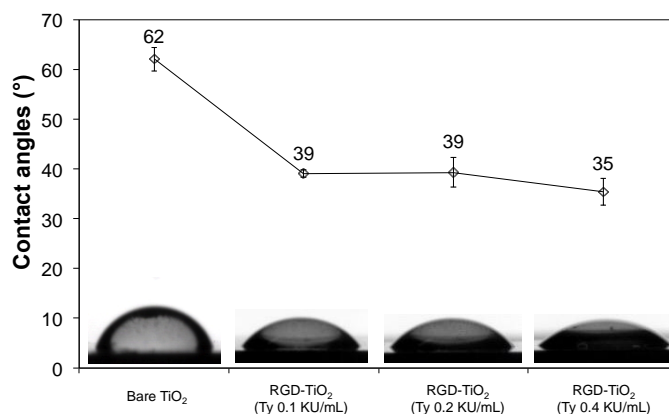


Fig. S3 Static water contact angles of bare TiO₂ and peptide functionalized TiO₂ via the tyrosinase-catalyzed reaction.

Quantification of immobilized peptide on TiO₂ surfaces: To determine the surface density of peptides, substrates were prepared using the GRGDGGGGGY (GRGD–Y) sequence due to the presence of the terminal primary amine group. The peptide density on the TiO₂ substrates was quantified using a fluorescamine assay.^[1] The GRGD–Y conjugated TiO₂ substrates were immersed in 375 μ L of PBS (0.01 M and pH 7.4) and 125 μ L of fluorescamine solution (4 mg/mL in acetone). After vigorous vortexing at RT for 1 min, the 200 μ L of reacted solutions were put into the 96 well plate, and then the fluorescence intensities of the substrates were measured using a multi-plate reader at an excitation and emission wave length of 390 nm and 475 nm equipped in the instrument. A standard curve was obtained from the intensity of known concentration of glycine (0–4 μ g/mL). The values of the RGD peptide concentration was estimated by using the equation of the standard curve, and the RGD peptide density on the substrates was calculated by the division of the RGD peptide concentration by the total surface area of the TiO₂ substrates.

Table S1. Quantitative analysis of XPS data for RGD–Y immobilized TiO₂ surfaces

Samples	C(1s)	O(1s)	N(1s)	N/C ratio
TiO ₂	37.87	58.43	3.69	0.10
RGD-TiO ₂	39.55	53.28	7.17	0.18

***In vitro* MC3T3–E1 culture:** The MC3T3–E1 cell was used to evaluate the *in vitro* cell attachment and spreading on the TiO₂ substrates. The cells were cultured on the surface of the TiO₂ surfaces at a density of 1×10^4 cells/cm² with α -MEM supplemented with or without 10% FBS including 1% PS under standard culture conditions (37 °C and 5% CO₂) for 24 h. The morphology of the cells cultured on the TiO₂ substrates was observed by fluorescence microscopy (TE 2000, Nikon, Japan). To observe the cell morphology, the cell nucleus and F-actin were stained with rhodamine-phalloidin and Hoechst 33258, respectively. For the cell staining, the samples were fixed with 4% formaldehyde for 30 min at RT. The cultured cells were permeabilized with a cyto-skeletal buffer solution for 20 min at 4 °C. The cells were blocked with 5% FBS including 0.1% Tween-20 for 1 h at 37 °C and then incubated with rhodamine-phalloidin and Hoechst 33258 for 2 h at 37 °C. After the samples were mounted on glass slides, the fluorescence images were obtained using fluorescence microscopy. Although it was possible to confirm the degree of cell attachment by MTT assay, the initial attachment and spreading of the cells in the surfaces was evaluated by using cytoskeleton-staining method to confirm the cell-to-material interaction.

Immobilization efficiency comparing dopamine (DA) with tyramine (TA) using tyrosinase system: Dopamine (DA) and tyramine (TA) were dissolved in a 0.01 M PBS solution (1 mg/mL, pH 7.4) and TiO₂ substrates were immersed into the solutions to produce polydopamine coated (PDA) surfaces. The reactions were performed with or without tyrosinase for 10 min. After the reaction, the substrates were washed using distilled water and dried in a vacuum oven. The surfaces of the modified substrates were characterized by static water contact angle and fluorescence observation. For the fluorescence observation, the PDA coated TiO₂ substrates were tagged using a FITC probe that can react with the primary amine on the modified surfaces. PDA coated substrates were immersed in 400 μ L of a FITC solution (2 mg/mL in ethanol) at RT for 6 h. After the reaction, the FITC tagged substrates were washed using distilled water and ethanol to remove unreacted FITC molecules. The fluorescence intensity was observed using a fluorescence microscopy with an excitation and emission wavelength of 395 nm and 521 nm, respectively.

Statistical Analysis: All data values were indicated as mean \pm standard deviation (SD). A student's t-test was used to assess statistical significance of the results ($*P < 0.001$).

Reference

1. K. M. Park, I. Jun, Y. K. Joung, H. Shin and K. D. Park, *Soft Matter*, 2011,7,986.