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Self-assembly and bioactivity of a polymer/peptide conjugate containing the RGD cell adhesion motif and PEG

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ABSTRACT

The self-assembly and bioactivity of the peptide–polymer conjugate DGRFFF–PEG3000 containing the RGD cell adhesion motif has been examined, in aqueous solution. The conjugate is designed to be amphiphilic by incorporation of three hydrophobic phenylalanine residues as well as the RGD unit and a short poly(ethylene glycol) (PEG) chain of molar mass 3000 kg mol^{-1}. Above a critical aggregation concentration, determined by fluorescence measurements, signals of β-sheet structure are revealed by spectroscopic measurements, as well as X-ray diffraction. At high concentration, a self-assembled fibril nanostructure is revealed by electron microscopy. The fibrils are observed despite PEG crystallization which occurs on drying. This suggests that DGRFFF has an aggregation tendency that is sufficiently strong not to be prevented by PEG crystallization. The adhesion, viability and proliferation of human corneal fibroblasts was examined for films of the conjugate on tissue culture plates (TCPs) as well as low attachment plates. On TCP, DGRFFF–PEG3000 films prepared at sufficiently low concentration are viable, and cell proliferation is observed. However, on low attachment surfaces, neither cell adhesion nor proliferation was observed, indicating that the RGD motif was not available to enhance cell adhesion. This was ascribed to the core–shell architecture of the self-assembled fibrils with a peptide core surrounded by a PEG shell which hinders access to the RGD unit.

1. Introduction

Peptide–polymer conjugates offer the potential to develop new materials with applications at the synthetic-biology interface. These materials combine the properties of synthetic polymers such as inexpensiveness and control of molecular weight with those of peptides which provide highly specific biofunctionality through precisely defined sequence design. A number of excellent reviews discuss different methods to synthesize polymer–peptide conjugates as well as their potential applications [1–11]. Conjugates containing poly(ethylene glycol) (PEG) have attracted considerable attention due to its ready availability/ease of synthesis, well-characterized physico-chemical properties and its biocompatibility. PEG is already used in protein–polymer conjugates on the market for applications in drug delivery [12–16].

Here, we investigate the self-assembly of the peptide–PEG conjugate DGRFFF–PEG3000 which contains the RGD cell adhesion motif from fibronectin [17,18], widely used in the development of synthetic materials for bioengineering, and the basis of our previous studies on the self-assembly of Fmoc-RGD(S) conjugates [Fmoc = *N*-(fluorenyl)-9-methoxycarbonyl] [19,20]. In order to modulate the amphiphilicity of the conjugate, three phenylalanine residues were incorporated to increase the hydrophobicity of the peptide. The phenylalanine units also serve as spacers between the functional RGD unit and the PEG chain. The self-assembly of PEG-FFFF conjugates has been examined by ourselves [21] and others [22,23]. Many groups have investigated cross-linked PEG hydrogels incorporating RGD-based cell adhesion motifs as cell-responsive biomaterials (see for example Refs. [24–28]). PEG/RGD-based

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peptide conjugates grafted to solid substrates have been studied as supports for cell growth and differentiation [29,30]. Despite this diverse research activity, there is little work to our knowledge, on non-crosslinked PEG conjugates comprising the RGD motif. Lutz and coworkers have reported the synthesis of poly(oligo(ethylene glycol) acrylate)-*b*-GGRGDG using ARTP and click methods [31]. Here, we examine a PEG–RGD (with backbone ethylene glycol units) conjugate, studying both solution self-assembly and the influence of PEG crystallization upon drying, as well as bioactivity via cell adhesion, viability and proliferation measurements. The conjugate is named DGRFFF–PEG consistent with our previous notation for peptide–PEG conjugates (and the convention that the *N* terminus is indicate on the left), although it can also be viewed as PEG-FFFRGD with the RGD unit presented at the *N* terminus.

2. Experimental

2.1. Materials

The conjugate was synthesized by Rapp Polymere GmbH using solid phase peptide synthesis methods, with Tentagel resins. The coupled PEG chain was analysed by GPC (1 mg/mL, THF eluent, on a PSS SDV 5 m linear S system) providing M_w = 3108 g mol^{-1}, M_n = 3015 g mol^{-1} and $Đ$ = 1.03. ES-MSI (NMI, Germany) provided M_n = 3700 g mol^{-1} for the DGRFFF–PEG conjugate, consistent with conjugation of DGRFFF (788 g mol^{-1}) to the PEG precursor. GPC (Chromolith RP18, 100-4,6 column) indicated a near monomodal molecular weight distribution.

To characterize self-assembly in water, solutions were made using amounts of peptide dissolved in Millipore water.

2.2. Fluorescence spectroscopy

Spectra were recorded with a Varian Cary Eclipse Fluorescence Spectrometer with samples in 4 mm inner width quartz cuvettes. Thioflavin T spectra were recorded from 460 to 600 nm using an excitation wavelength λ_{ex} = 440 nm. ThT assays were performed using a 4.0×10^{-3} wt.% ThT solution. Pyrene fluorescence assays were made using a sample containing only Pyr (1.3×10^{-5} wt.%), or a set of DGRFFF–PEG3000 solutions dissolved in 1.3×10^{-5} wt.% Pyr. All spectra were measured from 360 to 500 nm, using λ_{ex} = 339 nm.

2.3. Circular dichroism (CD)

Spectra were recorded using a Chirascan spectropolarimeter (Applied Photophysics, UK). CD was performed on a 2 wt.% solution of DGRFFF–PEG3000 in water which was placed in a cover slip cuvette (0.01 mm thick). Spectra are presented with absorbance $A < 2$ at any measured point with a 0.5 nm step, 1 nm bandwidth and 1 s collection time per step at 20 °C, taking four averages.

2.4. FTIR

Spectra were recorded using a Nexus-FTIR spectrometer equipped with a DTGS detector and a multiple reflection attenuated total reflectance (ATR) system. Solutions of DGRFFF–PEG3000 in D_2O (1 and 23 wt.%) were sandwiched in ring spacers between two CaF_2 plate windows (spacer 0.006 mm). All spectra were scanned 128 times over the range of 4000–950 cm^{-1}.

2.5. X-ray diffraction (XRD)

Measurements were performed on stalks prepared by drying filaments of the peptide from a 23 wt.% solution. The peptide solution was suspended between the ends of wax-coated capillaries and dried. The stalk was mounted (vertically) onto the four axis goniometer of a RAXIS IV++ X-ray diffractometer (Rigaku) equipped with a rotating anode generator. The XRD data was collected using a Saturn 992 CCD camera.

2.6. Polarized optical microscopy (POM)

A sample (23 wt.% peptide in water) was placed on a glass slide and dried. Images were obtained using an Olympus BX-41 optical microscope with an Olympus SP350 digital camera.

2.7. Transmission Electron Microscopy (TEM)

Imaging was performed using a Philips CM20 TEM microscope operated at 200 kV. Droplets of a 23 wt.% DGRFFF–PEG3000 solution were placed on Cu grids coated with a carbon film (Agar Scientific, UK), stained with uranyl acetate (1 wt.%) (Agar Scientific, UK) and dried.

2.8. Preparation of DGRFFF–PEG films for cell culture

Lyophilized DGRFFF–PEG3000 was solubilized in double distilled water at 0.01–0.4 wt.%. To produce film coatings, aliquots from solutions of the conjugate were spotted onto sterile tissue culture polystyrene (TCP) (Nunc, Thermo Fisher Scientific) or low-attachment (LA) plates (Costar, Corning Life Sciences) and dried overnight at room temperature inside an aseptic cell culture cabinet. The resulting films were washed three times with sterile phosphate buffer saline (PBS) just prior to cell seeding.

2.9. Cell culture

Human corneal fibroblasts were isolated from postmortem human corneal rings. Briefly, cornea tissue was shredded using a scalpel, transferred to Dulbecco's Modified Eagle's Medium (DMEM) containing 2 g L^{-1} of type-I collagenase and 5% FBS, incubated under rotation for 5 h at 37 °C, and further incubated with 0.25% Trypsin–EDTA in DMEM medium for 10 min. Isolated cells were cultured in polystyrene tissue culture flasks with DMEM/F12 media supplemented with 5% FBS, 1 mM ascorbic acid, and 1% penicillin/streptomycin, which was replenished every 2–3 days.

2.10. Cell adhesion, viability, and proliferation on DGRFFF–PEG3000 films

Third-passage cells were seeded at 1.5×10^4 cells cm^{-2} onto TCP or LA surfaces coated by the PA films. Cell number