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# Substrate chemistry influences the morphology and biological function of adsorbed extracellular matrix assemblies

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**Abstract**

In addition to mediating cell signalling events, native extracellular matrix (ECM) assemblies interact with other ECM components, act as reservoirs for soluble signalling molecules and perform structural roles. The potential of native ECM assemblies in the manufacture of biomimetic materials has not been fully exploited due, in part, to the effects of substrate interactions on their morphology. We have previously demonstrated that the ECM components, fibrillin and type VI collagen microfibrils, exhibit substrate dependent morphologies on chemically and topographically variable heterogeneous surfaces. Using both cleaning and coating approaches on silicon wafers and glass coverslips we have produced chemically homogeneous, topographically similar substrates which cover a large amphiphilic range. Extremes of substrate amphiphilicity induced morphological changes in periodicity, curvature and lateral spreading which may mask binding sites or disrupt domain structure. Biological functionality, as assayed by the ability to support cell spreading, was significantly reduced for fibrillin microfibrils adsorbed on highly hydrophilic substrates (contact angle 20.7°) compared with less hydrophilic (contact angle 38.3°) and hydrophobic (contact angle 92.8°) substrates. With an appropriate choice of surface chemistry, multifunctional ECM assemblies retain their native morphology and biological functionality.


*Keywords:* Extracellular matrix (ECM); Atomic force microscopy (AFM); Surface energy; Surface roughness; Protein adsorption; Cell spreading

## 1. Introduction

Many synthetic polymers used in tissue engineering applications are non-charged or weakly charged. UV irradiation to increase the surface energy of materials such as expanded polytetrafluoroethylene (ePTFE) and polyurethane in an $NH_3$ atmosphere has been employed to increase cellular adhesion [1]. Biodegradable polyesters such as poly(lactic-*co*-glycolic acid) (PLGA), which degrade to non-toxic lactic and glycolic acids, have also been widely used as tissue engineering scaffolds [2,3]. As with ePTFE, the cell adhesion properties of unmodified hydrophobic PLGA and related polylactide surfaces are poor and the physicochemical introduction of hydrophilic groups and the incorporation of cell adhesion peptides have been used to enhance cell attachment [4,5].

An alternative approach to enhancing cellular adhesion to synthetic substrates involves the design and manufacture of biomimetic materials in which adsorbed

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native extracellular matrix (ECM) proteins or ECM derived peptides influence cellular adhesion and phenotype [6]. Cellular adhesion to fibronectin and other ECM proteins, such as the macro-molecular assemblies formed by fibrillin and type VI collagen, is mediated by integrins which bind short ECM peptide sequences such as RGD (Arg-Gly-Asp) regulating cell adhesion, migration proliferation and secretory responses [7]. It has been proposed that the enhanced cellular adhesion observed on physicochemically modified hydrophilic PLGA surfaces may be mediated by increased adsorption of cell-binding ECM serum proteins such as fibronectin and vitronectin [4]. The ease of synthesis of short cellular recognition site peptide sequences has encouraged the study of synthetic peptides as potential biomimetic biomaterials [6]. Short peptide sequences however lack the diverse functionality of native ECM components which in vivo act as a reservoirs for soluble signalling molecules, bind other ECM proteins and perform important structural roles [8]. A major barrier to the use of native ECM proteins in the construction of biomimetic materials is substrate induced random folding which may sterically hinder binding events [6]. Regulation of cell phenotype in vitro is critically dependent on the establishment of appropriate cell to adsorbed ECM protein interactions. In turn these interactions require the preservation of native protein morphology which is dependent on the substrate [9–11].

Due to their amphiphilic nature proteins adsorb to most surfaces over a wide range of solution conditions [12]. Structural re-arrangements, which play an important role in the adsorption process, are known to influence the biological function of native ECM proteins, such as fibronectin and synthetic cell-adhesion peptides [6,13–15]. Steered molecular dynamic studies suggest that the substrate specific integrin binding of fibronectin may be a consequence of the relative movement of domains and the physical separation of the RGD (Arg-Gly-Asp) cellular recognition site and the adjacent synergy site [16]. In addition to changes in intra and inter-domain spacing (alterations in quaternary and tertiary structure), atomic force microscopy (AFM) force spectroscopy and circular dichroism (CD) studies suggest that the forces generated during protein/substrate interactions may be of sufficient magnitude to disrupt protein secondary structure [17,18].

Fibrillin and type VI collagen are modular ECM proteins which form structurally complex supra-molecular assemblies known as microfibrils. These naturally occurring multifunctional ECM components have the potential to produce highly effective biomimetic materials supporting cell attachment/spreading and linking cells to other ECM components [19–22]. We have previously demonstrated by AFM that fibrillin and type VI collagen microfibrils exhibit substrate dependent morphologies on surfaces which vary in chemistry, topography and the degree of chemical heterogeneity [11]. The chemical modification of substrates with thin polymer films or self-assembled monolayers provides a way of manufacturing topographically similar substrates which differ only in surface chemistry. Variations in surface chemistry and topography may influence adsorbed protein morphology and are known to affect contact angle estimates of substrate wettability [23]. Coating silicon oxide (SO) wafers with PLGA or octadecyltrimethoxysilane (OTS) produces chemically distinct homogeneous substrates with a wide amphiphilic range [2,24]. Similar substrate amphiphilicities may be induced on glass coverslips using combinations of alcohol/acid washes and vapour silanisation [25,26]. We have employed atomic force microscopy to investigate the effects of surface chemistry on fibrillin and type VI collagen microfibrils adsorbed on chemically homogeneous, topographically similar silicon wafer and glass substrates. The effect of substrate induced conformational changes on biologically important interactions was determined by cell spreading assays on fibrillin microfibril ligands adsorbed to chemically modified transparent glass coverslips.

## 2. Experimental section

### 2.1. Materials and reagents

Second trimester foetal calves and adult bovine eyes were obtained from the local abattoir within 1 h of death. Bacterial collagenase (type 1A), bovine serum albumin (BSA), phenylmethylsulfonyl fluoride (PMSF), *N*-ethylmaleimide (NEM), 10 nm gold colloids, poly-L-lysine (PLL), dichloromethane, hexadecane and Sigmacote® (chlorinated organopolysiloxane in heptane) were obtained from Sigma-Aldrich (Poole, Dorset, UK). Poly(DL-lactide-*co*-glycolide) (PLGA 50:50) with an average molecular weight of 50,000–75,000 and glass transition point of 45–50 °C and octadecyltrimethoxysilane (OTS) were also purchased from Sigma-Aldrich and used as supplied. Sepharose CL-2B was supplied by Amersham Biosciences UK Ltd. (Little Chalfont, Buckinghamshire, UK). Primary human dermal fibroblasts (HDF) were obtained from Cascade Biologics Inc. (Mansfield, Nottinghamshire, UK). DMEM/HEPES cell culture medium was obtained from Invitrogen Ltd. (Paisley, Renfrewshire, UK). Neutral Decon was obtained from Decon laboratories (Hove, East Sussex, UK). All other reagents were of analytical grade. Metal support stubs and Olympus high aspect ratio etched silicon probes (spring constant of $42\,N\,m^{-1}$) were obtained from Veeco Instruments (Santa Barbara, California, USA). Non-doped native silicon oxide wafers with $\langle 1\,1\,1 \rangle$ orientation were obtained from Compart Technology Ltd (Peterborough, Cambridgeshire, UK) and were used as supplied. Borosilicate glass coverslips (13 mm diameter) were obtained from Scientific Laboratory Supplies (Nottingham, Nottinghamshire, UK). Muscovite mica was obtained from Agar Scientific