Immunobiology 221 (2016) 1237–1246

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

The impact of surface chemistry modification on macrophage polarisation

Hassan M. Rostam[a], Sonali Singh[a], Fabian Salazar[a], Peter Magennis[b], Andrew Hook[b], Taranjit Singh[b], Nihal E. Vrana[c,d], Morgan R. Alexander[b], Amir M. Ghaemmaghami[a,*]

[a] Immunology and Tissue Modelling Group, Division of Immunology, School of Life Science, University of Nottingham, Faculty of Medicine & Health Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, UK
[b] Interface and Surface Analysis Centre, School of Pharmacy, University of Nottingham, UK
[c] Protip Medical, 8 Place de l'Hôpital, 67000 Strasbourg, France
[d] INSERM UMR 1121, 11 rue Humann, 67085 Strasbourg, France

ARTICLE INFO

Article history:
Received 8 February 2016
Received in revised form 1 June 2016
Accepted 10 June 2016
Available online 14 June 2016

Keywords:
Macrophage
Monocyte
M1
M2
Macrophage polarisation
Biomaterials
Surface chemistry
Oxygen plasma etching
Water contact angle
Foreign body response

ABSTRACT

Macrophages are innate immune cells that have a central role in combating infection and maintaining tissue homeostasis. They exhibit remarkable plasticity in response to environmental cues. At either end of a broad activation spectrum are pro-inflammatory (M1) and anti-inflammatory (M2) macrophages with distinct functional and phenotypical characteristics. Macrophages also play a crucial role in orchestrating immune responses to biomaterials used in the fabrication of implantable devices and drug delivery systems. To assess the impact of different surface chemistries on macrophage polarisation, human monocytes were cultured for 6 days on untreated hydrophobic polystyrene (PS) and hydrophilic O_2 plasma-etched polystyrene (O_2-PS40) surfaces. Our data clearly show that monocytes cultured on the hydrophilic O_2-PS40 surface are polarised towards an M1-like phenotype, as evidenced by significantly higher expression of the pro-inflammatory transcription factors STAT1 and IRF5. By comparison, monocytes cultured on the hydrophobic PS surface exhibited an M2-like phenotype with high expression of mannose receptor (MR) and production of the anti-inflammatory cytokines IL-10 and CCL18. While the molecular basis of such different patterns of cell differentiation is yet to be fully elucidated, we hypothesise that it is due to the adsorption of different biomolecules on these surface chemistries. Indeed our surface characterisation data show quantitative and qualitative differences between the protein layers on the O_2-PS40 surface compared to PS surface which could be responsible for the observed differential macrophage polarisation on each surface.

1. Introduction

Implanted biomaterials typically trigger an inflammatory immune response orchestrated by macrophages (Higgins et al., 2009). Often this results in a cascade of inflammatory and fibrotic events known as the foreign body response (FBR) (Bartoli and Godleski, 2010). FBR begins with protein adsorption on the implant surface, which promotes the adhesion of monocytes and macrophages (Shen et al., 2004). Macrophages are sensitive to microenvironmental changes and mount a rapid response to implanted materials. They can also fuse under the influence of the cytokines interleukin 4 (IL-4) and IL-13, forming foreign body giant cells (FBGCs). Macrophages and FBGCs induce infiltration and stimulation of immune cells (e.g. lymphocytes) and stromal cells (e.g. fibroblasts), leading to inflammation and fibrosis at the implant site (Rostam et al., 2015). FBR can end with sequestration of the implant within a fibrous capsule (Anderson et al., 2008). This creates mechanical and functional problems, and for devices such as electrodes, can mean the end of their functional life (Morais et al., 2010).

Macrophages are extremely plastic cells, adopting a wide spectrum of phenotypes in response to different stimuli (Sica and Mantovani, 2012). The physical, chemical, and topographical characteristics of implanted materials can affect macrophage polarisation, resulting in macrophages that are either predominantly pro-inflammatory or anti-inflammatory (Rostam et al., 2015).

Abbreviations: O_2-PS, oxygen plasma etched polystyrene; PS, polystyrene; RGD, arginine-glycine-aspartate; TCP, tissue culture plastic; WCA, water contact angle.

* Corresponding author.
E-mail address: amir.ghaemmaghami@nottingham.ac.uk (A.M. Ghaemmaghami).

http://dx.doi.org/10.1016/j.imbio.2016.06.010

Table 1
Forward and reverse primer sequences used for qRT-PCR.

Genes	Primers/probe	Sequence (5′–3′)
GAPDH	Forward	GAGTCAACGGATTTGGTCGT
	Reverse	GACAAGCTTCCCGTTCTCAG
STAT1	Forward	GGAAGGGGCCATCACATTCA
	Reverse	GTAGGGTTCAACCGCATGGA
SOCS1	Forward	CCCTGGTTGTTGTAGCAGCTT
	Reverse	TTGTGCAAAGATACTGGGTATATGT
IRF5	Forward	GCCATGAGCAGGGAAAGAAC
	Reverse	CCCTTAGGCAATTCCTCCTATACA
SOCS3	Life Technologies Hs02330328_s1 *(Taqman)*	
IRF4	Life Technologies Hs01056533_m1 *(Taqman)*	

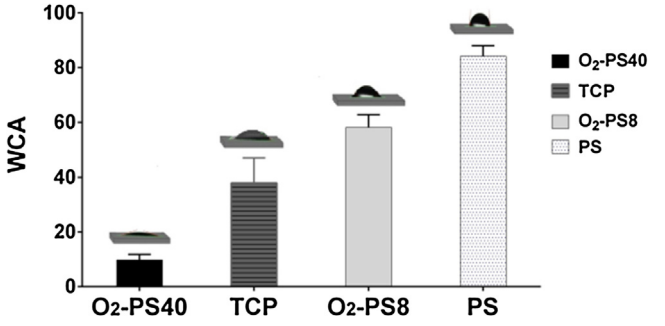

Fig. 1. Water contact angle (WCA) of polystyrene and TCP surfaces. The graph depicts the mean WCA ± SD for n = 4 oxygen plasma-etched polystyrene (O_2-PS40 and O_2-PS8), tissue culture plastic (TCP), and untreated polystyrene (PS) surfaces in ascending order of WCA.

The two best studied macrophage phenotypes are M1 and M2. M1 (classically activated) macrophages with pro-inflammatory and anti-tumour function (Sutterwala et al., 1997) can be generated *in vitro* from monocytes by treatment with the T helper (TH) 1 cytokine interferon gamma (IFN-γ) (Garcia et al., 2014) and/or lipopolysaccharide (LPS)(Mills et al., 2000). The addition of granulocyte macrophage colony-stimulating factor (GM-CSF) during M1 polarisation augments the pro-inflammatory function of these cells (Hamilton, 2002; Hamilton, 2008). By contrast, M2 (alternatively activated) macrophages with anti-inflammatory and pro-wound healing activities (Sutterwala et al., 1997) can be generated *in vitro* from monocytes by treatment with the TH2 cytokines IL-4 (Garcia et al., 2014; Verreck et al., 2004) and/or IL-13 (Garcia et al., 2014). The addition of macrophage colony-stimulating factor (M-CSF) during M2 polarisation can enhance the anti-inflammatory function of M2 macrophages (Garcia et al., 2014; Verreck et al., 2004).

M1 macrophages produce high levels of pro-inflammatory cytokines such as IL-12, IL-23 (Mantovani et al., 2004), tumour necrosis factor alpha (TNF-α) (Hofkens et al., 2011; Hao et al., 2012), IL-6, and IL-1β (Hofkens et al., 2011). They are also characterised by elevated expression of the chemokine (C-C motif) receptor 7 (CCR7)(Agrawal, 2012), CCR2 (Willenborg et al., 2012), calprotectin (Bartneck et al., 2010), and nitric oxide synthase 2, inducible (NOS2) (Edin et al., 2012). In contrast, M2 macrophages secrete large amounts of anti-inflammatory and pro-fibrotic cytokines such as IL-10 (Mantovani, 2006), transforming growth factor (TGF-β) (Hao et al., 2012), and IL-1 receptor antagonist (IL-1RA) (Baitsch et al., 2011). In addition, these cells express high levels of mannose receptor (MR) (Agrawal, 2012; Mantovani, 2006; Choi et al., 2010) and the scavenger receptor CD163 (Edin et al., 2012; Mantovani, 2006).

Additionally, M1 macrophages express high levels of prostaglandin-endoperoxide synthase 2 (Ptgs2 or Cox2) and IL23p19 genes, and exhibit phosphorylation of signal transducer and activator of transcription 1 (STAT1). M2 macrophages can be identified by high levels of Kruppel-like factor 4 (Klf4) and chitinase 3-like 2 (Chi3l2 or Ykl39) gene expression, and STAT6 phosphorylation (Murray and Wynn, 2011).

Appropriate regulation of macrophage activation post-implantation is extremely important, since these cells play a crucial role in the elimination of microbes and debris, biodegradation, tissue regeneration and vascularisation, and extracellular matrix reorganisation following tissue damage (Xia and Triffitt, 2006). Therefore, macrophages and FBGCs, either directly or through modulating the function of other cell types, can tip the balance between chronic inflammation and resolution/wound healing following biomaterial implantation (Solheim et al., 2000).

In order to minimise implant-associated inflammation, various approaches have been used to modulate macrophage-biomaterial interactions (Rostam et al., 2015; Zaveri et al., 2010a, b). Biomaterial surface chemistry is one factor that impacts cellular responses (Unadkat et al., 2011) as it influences the amount, identity and conformation of protein adsorption on the surface (Sigal et al., 1998), which in turn modulates cell behaviour. For instance, surfaces functionalised with the arginine-glycine-aspartate (RGD) peptide, chitosan, and vitronectin stimulate expression of CD147, CD98, MR, and CD13 (molecules related to macrophage fusion) in monocytes (McNally and Anderson, 2015; Dadsetan et al., 2004; Brodbeck et al., 2002).

Modification of material surface chemistry has been used to change the functional properties and phenotype of different cell types (Murphy et al., 2014; Celiz et al., 2015), including immune cells (Sun et al., 2007; Senaratne et al., 2006). Such strategies would enable the development of materials with cell-instructive properties that could be used for devices such as pacemakers (Taguchi et al., 2014), prosthetic joints (Katti, 2004), intraocular lenses (McCoy et al., 2012), vascular grafts (Xue and Greisler, 2003) and degradable sutures (Cao and Wang, 2009).

In this study, we employed plasma etching, a process used routinely in the mass production of tissue culture ware (Zamora et al., 2003), to develop different surface chemistries using polystyrene as our substrate. We then characterised the phenotype, cytokine profile and functional properties of human monocytes that were cultured on these surfaces for 6 days. Finally, to better understand how these surface chemistries influence monocyte differentiation and macrophage polarisation, we conducted initial characterisation of protein adsorbates on each of the surfaces.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma-Aldrich unless otherwise stated.

2.2. Surface preparation

Polystyrene samples (2 cm^2) were made by cutting untreated polystyrene (PS) petri dishes (Greiner bio-one Ltd.). Two oxygen plasma etched polystyrene (O_2-PS) surfaces were made by etching untreated PS with O_2 plasma using radio frequency powered equipment described previously (Majani et al., 2010); these were: 1) O_2-PS40 - 40 W, 300 mTorr, 60 s, and 2) O_2-PS8–8W, 300 mTorr, 5 s. Polystyrene tissue culture plates (TCP) (Corning), which have a proprietary treatment, were used as the fourth surface chemistry.