Biomaterials 52 (2015) 88–102

Contents lists available at ScienceDirect

Biomaterials

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

Development and functional evaluation of biomimetic silicone surfaces with hierarchical micro/nano-topographical features demonstrates favourable *in vitro* foreign body response of breast-derived fibroblasts

Daniel J.T. Kyle [a, b], Antonios Oikonomou [b], Ernie Hill [b], Ardeshir Bayat [a, *]

[a] *Plastic and Reconstructive Surgery Research, Manchester Institute of Biotechnology, University of Manchester, Manchester, UK*
[b] *School of Computer Science, Centre for Mesoscience and Nanotechnology, The University of Manchester, Manchester, UK*

A R T I C L E I N F O

Article history:
Received 31 July 2014
Received in revised form
25 January 2015
Accepted 1 February 2015
Available online

Keywords:
Biomimetic topography
Micro and nano patterning
Cell-implant interface
Acellular dermal matrix (ADM)
Silicone breast implant
Capsular contracture

A B S T R A C T

Reproducing extracellular matrix topographical cues, such as those present within acellular dermal matrix (ADM), in synthetic implant surfaces, may augment cellular responses, independent of surface chemistry. This could lead to enhanced implant integration and performance while reducing complications. In this work, the hierarchical micro and nanoscale features of ADM were accurately and reproducibly replicated in polydimethylsiloxane (PDMS), using an innovative maskless 3D grayscale fabrication process not previously reported. Human breast derived fibroblasts ($n = 5$) were cultured on PDMS surfaces and compared to commercially available smooth and textured silicone implant surfaces, for up to one week. Cell attachment, proliferation and cytotoxicity, in addition to immunofluorescence staining, SEM imaging, qRT-PCR and cytokine array were performed. ADM PDMS surfaces promoted cell adhesion, proliferation and survival ($p = < 0.05$), in addition to increased focal contact formation and spread fibroblast morphology when compared to commercially available implant surfaces. PCNA, vinculin and collagen 1 were up-regulated in fibroblasts on biomimetic surfaces while IL8, TNFα, TGFβ1 and HSP60 were down-regulated ($p = < 0.05$). A reduced inflammatory cytokine response was also observed ($p = < 0.05$). This study represents a novel approach to the development of functionalised biomimetic prosthetic implant surfaces which were demonstrated to significantly attenuate the acute *in vitro* foreign body reaction to silicone.

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Surgical implants provide a diverse variety of site-specific tissue replacements for a number of functions, which are available to the practising surgeon. Common examples in use today include breast implants, dental implants, nerve conduits, vascular implants and orthopaedic implants [1]. The increasing demand for synthetically engineered body implants is a result of an ageing population and the associated tissue degeneration and malignancy [2]. This trend will continue until tissue regeneration techniques utilising autologous mesenchymal stem cells to engineer tissue-specific replacements becomes perfected and available to routine clinical practice. The biomaterials industry is expected to be worth $58.1 billion in 2014 [3] as medical devices such as breast implants are being increasingly required, with 385,813 breast augmentations/ reconstructions (72% silicone implants) performed in the United States alone in 2013, up 2% from 2012 to 32% from 2000 [4].

However, current commercially available silicone mammary implants are not without their complications. For instance, silicone mammary implant surfaces suffer from significant limitation due to the formation of a constrictive fibrotic capsule post-implantation, known as capsular contracture, which results in firmness, deformity and pain in addition to device failure [5]. Capsular contracture formation remains the most common complication associated with silicone mammary implants, with rates ranging between 14.8 and 20.5% [6].

The exact pathoetiology of breast implant surface-related capsular contracture formation remains unclear, however, there

* Corresponding author. Manchester Institute of Biotechnology, University of Manchester, 131 Princess Street , Manchester, M17 DN, UK. Tel.: +44 161 306 5177.
E-mail address: Ardeshir.Bayat@manchester.ac.uk (A. Bayat).

http://dx.doi.org/10.1016/j.biomaterials.2015.02.003
0142-9612/© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

are a number of known risk factors, such as peri-prosthetic bacterial infection and radiotherapy pre or post-implantation, while submuscular placement and importantly, textured as opposed to smooth implants, can reduce contracture risk [7]. In addition to currently practised methods of limiting rates of capsular contracture formation through minimising bacterial infection around the implant (exogenous hypothesis), an alternative approach to this clinically significant complication is necessary to reduce the over-exaggerated foreign body reaction to the implant (endogenous hypothesis) [8].

Current commercially available silicone breast implants were fundamentally designed in the 1960's and the elastomeric implant shell (the site of the tissue/implant interface) is described as being either ‘smooth’ or ‘textured’ [9,10]. Textured implants are macroscopically rough surfaces formed either of nodular features or cuboid shaped pits, which are hundreds of microns large [11]. However, current silicone breast implants are intrinsically limited in their performance as the elastomeric shells (surfaces) were not designed with consideration of the mechanisms which promote favourable host response being a primary objective of the implants' function and rather they evolved through “trial and error optimization” [12], resulting in minimal advancement in implant performance. Conversely, due to the availability of advanced fabrication techniques and increased understanding of the variables which influence host-response at the cell-implant surface interface, medical devices manufactured today should aim to be non-toxic and non-immunogenic, whilst performing an active role in host response [13,14].

Micrometric and nanometric surface topographies influence cell attachment, proliferation, migration and differentiation in numerous cell types and on various substrates, both *in vitro* and *in vivo* [15–20]. It has been suggested that initial implant cell attachment and subsequent cytokine release may dictate the extent and impact of the foreign body reaction and clinical outcome through cell mechanotransduction and signal transduction mechanisms, which mediate cytokine/chemokine release and extracellular matrix (ECM) deposition [21–23]. This is the rationale behind the current tendency to use acellular dermal matrix (ADM) in implant-based breast augmentation/reconstruction. It is thought that ADM, in addition to providing inframammary support to the implant, provides peri-implant cells and tissues with a familiar biological microenvironment, containing complex nano and micro-topographical cues which delivers stimulus for specific and directed cell response [24–26]. The result is diminished foreign body reaction to the implant and reduced capsular contracture formation [27].

Our intuitive aim here was to design and fabricate a novel biomimetic silicone surface, acellular dermal matrix polydimethylsiloxane fabricated (ADM PDMS F), through reproduction of the complex hierarchical, nano and micro-scale topography of ADM using an innovative maskless 3D grayscale lithography fabrication process, not reported to date. This creative approach to implant surface design combines our understanding of the potential positive effects of micro and nanoscale topographies on cell function with the unique and inherent benefits of biomimetic structures. A silicone surface prepared using a direct casting (soft lithography) technique, acellular dermal matrix polydimethylsiloxane cast (ADM PDMS C), was also created to use as further validation.

Therefore, it is proposed that the biomimetic topographical cues of ADM in silicone may mitigate the acute *in vitro* foreign body response of breast-derived fibroblasts (BDFs) in comparison to commercially available smooth and textured silicone implant surfaces. For validation, a comprehensive *in vitro* biological evaluation of BDF cell function on respective surfaces through analysis of cell attachment, proliferation, morphology, gene expression and cytokine secretion was performed, to elucidate whether early BDF response to silicone surfaces could be positively altered through biomimetic topography.

2. Materials and methods

Tissue samples used in this study were obtained by the Plastics and Reconstructive Surgery Research (PRSR) Skin and Tissue Bank (North West Research Ethics Committee, Ethics code – 11/NW/0683). Patients were recruited and samples obtained following informed consent prior to elective breast reduction (to obtain skin and breast tissue). Patient demographics are found in Supplementary Table S1. Samples were anonymised and coded prior to use. In this work, two polydimethylsiloxane (PDMS) surfaces were fabricated, characterised and biologically evaluated; acellular dermal matrix polydimethylsiloxane fabricated surface (ADM PDMS F) and acellular dermal matrix polydimethylsiloxane cast surface (ADM PDMS C).

2.1. Collection of skin samples and tissue decellularisation protocol

Normal skin ($n = 3$) was collected from patients undergoing breast reduction surgery and stored in growth media containing Dulbeccos Modified Eagle Medium (DMEM) (Sigma-Aldrich, UK) supplemented with 10% fetal bovine serum (FBS) (PAA, Austria), penicillin (100 units/ml), streptomycin (100 units/ml) and L-glutamine (2 mM) (PAA Austria) while transported to laboratory. The skin was washed thoroughly in sterile phosphate buffered saline (PBS) supplemented with penicillin (100 units/ml) and streptomycin (100 units/ml), to remove any remaining blood, before hair and subcutaneous fat was removed. The decellularisation protocol followed is outlined in Hogg (2013) [28] and an exhaustive list of reagents used and exact protocol can be found in Supplementary Table S2 and S3, in addition to Hogg (2013) [28]. Briefly, the protocol involved moving the tissue through a succession of reagents, beginning with removal of the epidermis, followed by tissue disinfection in peracetic acid, digestion of cellular components in a hypotonic buffer, a detergent wash to remove cellular debris and removal of nuclear contents [28].

2.2. Immunohistochemistry and haematoxylin and eosin staining of ADM

Immunoperoxidase staining was performed for collagen type IV, collagen type VII and laminin V to confirm the presence of an intact basement membrane (BM), using Novacastra™ Novolink™ Polymer Detection System (Leica Biosystems, UK), following manufacturer's instructions. Briefly, formalin fixed, paraffin embedded tissue samples were serially sectioned into 5 μm thickness, mounted onto charged slides (Thermo Scientific, USA) and left to dry overnight. Sections were then deparaffinised, rehydrated and antigen retrieved in sodium citrate buffer (Ph 6 and 60 °C for 1 h). Endogenous peroxidase and non-specific protein binding was blocked and the section treated with primary antibodies overnight at 4 °C (Supplementary Table S4). The sections were then further incubated with Post Primary Block and then with Novalink Polymer before proteins were finally detected using DAB chromogen substrate buffer for peroxidase detection and counterstained with haematoxylin. In addition, sections were stained with haematoxylin and eosin (H&E), to ensure morphology of tissue was not altered by decellularisation protocol, using standard laboratory protocol as previously described [29]. All sections were imaged on an upright Olympus Microscope (BX51, Olympus, UK).

2.3. Fabrication of ADM PDMS F

2.3.1. Characterising ADM using atomic force microscopy (AFM)

ADM samples were placed basement membrane (BM) side up onto microscope slides and allowed to slowly air dry at 4 °C for 24 h. ADM was imaged using a Bruker Dimension Icon® Atomic Force Microscope (AFM) (Bruker, UK). Samples were imaged using ScanAsyst™ Air probes (Bruker, UK) (silicon nitride, nominal $k = 0.4$ N/m) and conducted in ScanAsyst™ mode. Peak Force Tapping™ (PFT) amplitude was 150–100 nm, and PFT frequency was 1 kHz. Scan rate was 0.5 Hz. A large, intact area of ADM (500 um) was imaged through obtaining numerous 90×90 μm^2 AFM scans using offsets in the X and Y direction (Supplementary Fig. S1A). Scans were performed in at least 3 different areas of the ADM sample and on 3 different patient samples.

2.3.2. Creating a grayscale ADM pattern for exposure

An ADM montage was created using the stitching feature within Mountain Maps® 7 imaging software (Digital Surf©, France) to stitch together numerous, adjacent raw 90×90 μm^2 AFM images of ADM. The montage was produced using X and Y offsets and similarities in height at the image edge to correctly align images, thereby forming a large intact area of ADM without leaving stitch lines (Supplementary Fig. S1C–D). The ADM montage was converted to an 8-bit grayscale image, consisting of 256 grayscale levels (Supplementary Fig. S1B–D), using the open source scanning probe analysis software Gwyddion (http://gwyddion.net/) which could then be read by a laser lithography system.

Download English Version:

https://daneshyari.com/en/article/6485986

Download Persian Version:

https://daneshyari.com/article/6485986

Daneshyari.com