Biomaterials 30 (2009) 2015–2022

Contents lists available at ScienceDirect

Biomaterials

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

The use of combinatorial topographical libraries for the screening of enhanced osteogenic expression and mineralization

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ARTICLE INFO

Article history:
Received 17 November 2008
Accepted 26 December 2008
Available online 28 January 2009

Keywords:
Biocompatibility
Cell culture
Microstructure
Surface topography

ABSTRACT

Nano- and microstructured surfaces are known to impact on the binding and differentiation of cells, but the detailed basic understanding of the underlying regulatory mechanisms is still scarce, which impedes the rational design of smart biomaterials. Towards a comprehensive analysis of the interplay between topographical parameters such as feature design and lateral and vertical dimensions we here report on a combinatorial screening approach, BioSurface Structure Array (BSSA) of test squares each with a distinct topography. Using such BSSA libraries of 504 topographically distinct surface structures, we have identified combinations of size, gap and height of structures which enhance mineralization as well as the expression of osteogenic markers of a preosteoblastic murine cell line. This generic BSSA screening platform is a versatile technology for the systematic identification of surfaces with specific biological properties, and it may for example be useful for optimizing the design of biomaterials for regulating cellular behaviour.

1. Introduction

In the past it has been shown that when mammalian cells bind to surfaces, the detailed surface topography influences the cell behaviour with respect to processes such as adhesion, orientation, differentiation, proliferation, changes in contact guidance, cytoskeletal organisation, focal adhesion point organization, apoptosis, macrophage activation, and gene expression [1–11]. Several papers have indicated that phosphorylation, expression, and translocation of transcription factors are factors that play a major role in the cellular response when cells are growing on micro- and nanostructured surfaces. More specifically, it has been shown that rat calvarial osteoblasts differentiate and mineralize on microstructured surfaces, resulting in the phosphorylation of Src, focal adhesion kinase (FAK), and ERK1/2 concomitant with activation through translocation of the transcription factor Runx2 from the cytoplasm to the nucleus [12–14]. Some of these cellular responses may be initiated through an alteration of the cellular contact with the microstructured surface as demonstrated for example by Biggs and co-workers [15], who seeded primary human osteoblasts upon substrates with groves/ridges varying in width from 10 to 100 μm and observed distinct differences in focal adhesion complex formation. Also, for mesenchymal stem cells (MSC) it has been demonstrated that surfaces with nanoscale features promote the differentiation of MSC without the use of growth factors [15–18]. Furthermore, Dalby et al. [17] have observed that pathways of gene regulation towards the mineralization of osteogenic cells are strongly affected by the detailed nanostructure of surfaces.

It thus appears that although some mechanistic insights have been obtained into the response of cell adhesion to surface topography, we are still far from a level of understanding that allows for the rational design of improved biomaterials with a predicted influence on cellular behaviour, or for the design of surfaces for stem cell propagation in the absence of feeder cells. Today many surfaces applied in cell adhesion studies are often still selected from a simple trial and error approach.

Here we present a BioSurface Structure Array (BSSA) platform technology enabling the systematic screening of cellular responses to a large variety of nano- and microstructured surfaces. In the present setting, each BSSA screening wafer was subdivided into 169 squares, each of which covered 3 mm × 3 mm on the array. With a typical cell size of 50 μm × 50 μm, each square contained up to 3600 cells, which enables statistical data analysis from each

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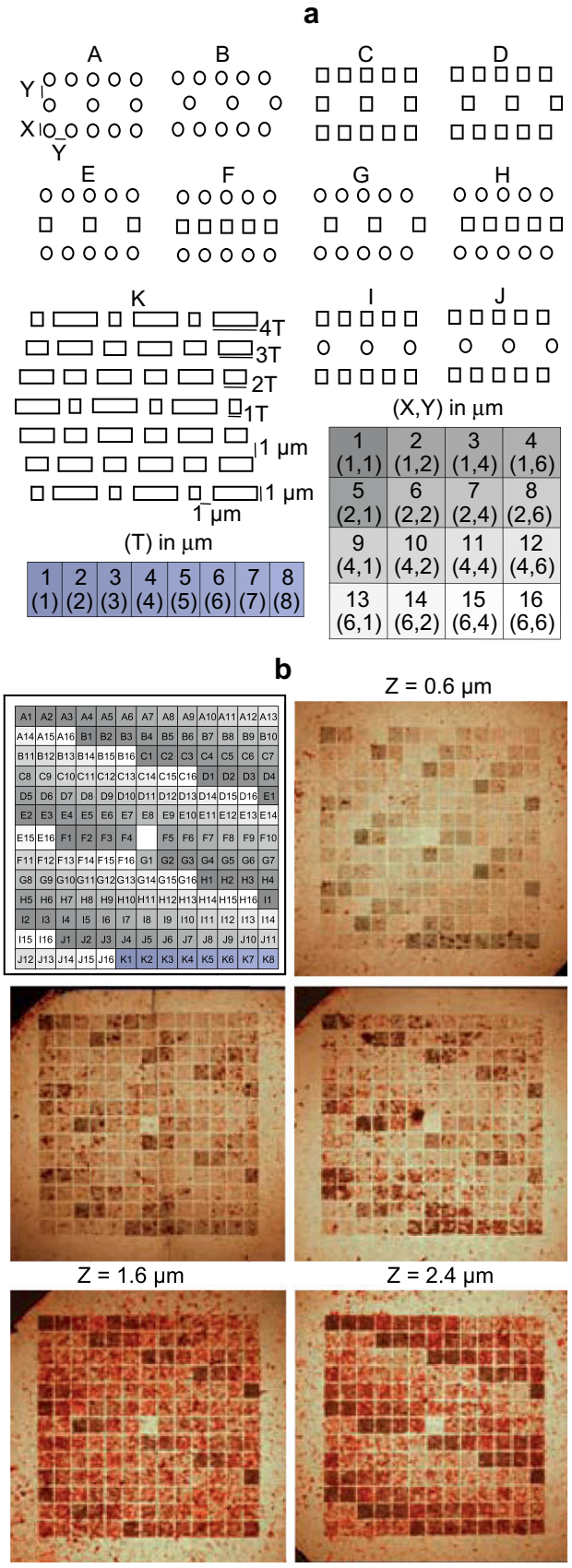
doi:10.1016/j.biomaterials.2008.12.081


a
A
B
C
D
E
F
G
H
K
I
J
Y
X
4T
3T
2T
1T
1 µm
1 µm
1 µm
(T) in µm
1 (1) 2 (2) 3 (3) 4 (4) 5 (5) 6 (6) 7 (7) 8 (8)
(X,Y) in µm
1 (1,1) 2 (1,2) 3 (1,4) 4 (1,6)
5 (2,1) 6 (2,2) 7 (2,4) 8 (2,6)
9 (4,1) 10 (4,2) 11 (4,4) 12 (4,6)
13 (6,1) 14 (6,2) 15 (6,4) 16 (6,6)
b
Z = 0.6 µm
Z = 1.6 µm
Z = 2.4 µm
c
Mineralization Height 1.6 µm
Mineralization Height 2.4 µm
Series A,C,E,I (2,3,15,16)
Series B,D,G,J (2,3,15,16)
Series F,H (2,3,15,16)
K 1-8
Control
d
Relative mineralization
Z=1.6 µm
Z=2.4 µm
Sharkskin
1.1 1.2 1.4 1.6 2.1 2.2 2.4 2.6 4.1 4.2 4.4 4.6 6.1 6.2 6.4 6.6
Control