Bioactive Materials 3 (2018) 355–369

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/en/journals/bioactive-materials/

Micro and Nanofabrication methods to control cell-substrate interactions and cell behavior: A review from the tissue engineering perspective

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ARTICLE INFO

Article history:
Received 30 December 2017
Received in revised form
9 May 2018
Accepted 10 May 2018

Keywords:
Microfabrication
Micropattern
Cell-material interaction
Differentiation
Cell adhesion

ABSTRACT

Cell-substrate interactions play a crucial role in the design of better biomaterials and integration of implants with the tissues. Adhesion is the binding process of the cells to the substrate through interactions between the surface molecules of the cell membrane and the substrate. There are several factors that affect cell adhesion including substrate surface chemistry, topography, and stiffness. These factors physically and chemically guide and influence the adhesion strength, spreading, shape and fate of the cell. Recently, technological advances enabled us to precisely engineer the geometry and chemistry of substrate surfaces enabling the control of the interaction cells with the substrate. Some of the most commonly used surface engineering methods for eliciting the desired cellular responses on biomaterials are photolithography, electron beam lithography, microcontact printing, and microfluidics. These methods allow production of nano- and micron level substrate features that can control cell adhesion, migration, differentiation, shape of the cells and the nuclei as well as measurement of the forces involved in such activities. This review aims to summarize the current techniques and associate these techniques with cellular responses in order to emphasize the effect of chemistry, dimensions, density and design of surface patterns on cell-substrate interactions. We conclude with future projections in the field of cell-substrate interactions in the hope of providing an outlook for the future studies.

1. Introduction

In the history of the biomaterials field since the ancient Egyptians, the achievement of biocompatibility was the main concern because it is the most critical property for a typical biomaterial. As the scientific and technological tools evolved it became evident that the architecture and topography of an implant is just as central to the implant design as the chemistry. The ultimate goal of research on cell-substrate interactions is to study the relationship between the substrate surface and the cell response it evokes. There are numerous studies involving substrates with different topography and chemistry, targeting different tissues, cells and cell responses. However, there is still no universal rule of thumb applicable to all situations. The outstanding question in implant surface design is to identify the best design for a target tissue and how it can be achieved with the current knowledge base. The proper approach would be the determination of ideal surface properties for each specific application while taking into consideration the contribution of surface topography to the performance of the implant. Today, with the vast variety of the scientific tools available, response of a cell to any stimulus can be controlled and studied in detail.

Cell shape, adhesion, migration, and fate are controlled by the properties of the substrate. These are topography (the surface architecture), stiffness, and bioactive cell adhesive cues such as peptides and proteins. In 1912, Harrison was first to show the effect

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Peer review under responsibility of KeAi Communications Co., Ltd.
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https://doi.org/10.1016/j.bioactmat.2018.05.005

of solid support materials on cell movement and morphogenesis by using spider web as the substrate [1]. He demonstrated that the cell shape and migration were correlated with the substrate organization and topography. Later, Paul Weiss (1947) showed that cells move and migrate by contact guidance [2]. Curtis and Varde were the first researchers to take advantage of these findings and employed topographical cues for the control of cell behavior [3]. The microfabrication techniques, initially developed for the electronic industry, came into use for the study of the behavior of cells on micro- and nanopatterned surfaces nearly three decades ago [4]. Since then, many studies were conducted to elucidate cell-substrate interactions on engineered surfaces prepared using many different materials, architectures and cells [5–21]. All of these studies showed that cellular functions are affected and in some cases improved by the substrates mimicking the extracellular matrix (ECM) topography. The substrate is not just a cell support but also a guide for adhesion, proliferation, morphology, and spreading by providing physical and chemical signals [11,22]. Substrate topography can affect the cellular functions differently depending on the cell type, pattern size and geometry, stiffness and chemical properties of the substrate material [20]. Sub-micron- to -nanoscale topographies affect cells directly since they have the similar size with ECM proteins such as fibronectin, collagen and laminin. These tissue subunits contain a large number of cells so, sub-millimeter topography can also affect cell-cell interactions, cell-cell signaling, and other cellular activities [23]. In order to understand the influence of surface topography on shape and other properties of a cell, the cell adhesion and mechanotransduction should be studied thoroughly.

Although the relationship between substrate topography and cell behavior has been studied by many authors, the main difficulty in this area is the abundance and complexity of the substrates which have different mechanical properties, sizes, shapes, distribution and chemistry of topographical cues in addition to the cell and tissue types used. There are several reviews in the literature which focus on topography especially concentrating on feature size and tissue type [24,25]. There are others on production methods of substrates [26–28], on the biological aspects of the interaction [29,30] and on the comparison of physical and chemical cues [31,32]. The current review aims to provide an integrative perspective on the biology, production methodology and the cell-topography relationships presented according to the cellular responses evoked with special emphasis on feature size.

This review summarizes the cell adhesion process in order to provide a framework to understand the mechanisms of cell-substrate interaction in the context of tissue engineering and the methods for fabricating such substrates. It provides a comprehensive survey of the current literature to reveal the role of surface pattern chemistry, dimensions, density and design on cell adhesion, alignment, migration, differentiation. It also evaluates nano- and microfabricated substrates as tools for controlling cell and nuclear shape, as well as measurement of the cellular forces.

2. Cell adhesion

Adhesion is a fundamental cellular process in tissue formation. It is about the binding of a cell to the extracellular matrix, surface or another cell through use of certain surface proteins [33]. This binding achieved through cell adhesion proteins, results in two particular mechanisms for the intracellular signal generation: originating a force on cytoskeletal elements which is transmitted throughout the cytosol to the nuclear lamina, and activation of signaling pathways and messengers [34]. These two processes are not mutually exclusive. Binding to a surface via adhesion molecules and transmission of a signal to the cytoskeleton involves a specific process called “focal adhesion”. Focal adhesion points are intersection nodes where the environmental mechanical signals received are transduced to intracellular forces and chemical signals through cytoskeletal connections and signaling proteins [35]. After the generation of a traction force on the cytoskeleton, this force is transmitted by the cytoskeletal elements to various structures in the cell in which nucleus holds distinct importance [36]. Following cell adhesion, many cellular events, including differentiation, apoptosis, and changes in the gene expression profiles, are indirectly affected from the forces generated on nuclear lamina [36,37].

Cells adhere to each other, to ECM or any substrate with the structures called cell junctions which are tight junctions (*zonula occludens),* intermediate junction (*zonula adherens*), and desmosomes (*macula adherens*). *Zonula occludens* (tight junction) is formed by the fusion of adjacent cell membranes, *zonula adherens* (intermediate junction) is a ~200 Å intercellular space occupied by homogeneous amorphous material, and *macula adherens* (desmosome) is a ~240 Å intercellular space with a central dense disc [38]. Adhesion of cells to substrates was first studied using the interference reflection microscopy, and showed that cell-substrate interactions took place at the adhesions (100 Å) while rest of the cell surface was further away [39]. It was shown that a surface treatment that generates hydroxyl groups on polystyrene resulted in enhanced cell attachment [40]. Therefore it was proven that for the cells to adhere to a surface, there must be specific chemical groups available on the substrate [40]. This was later explained by the presence of specific cell surface proteins called Cell Adhesion Molecules (CAMs) [41]. These molecules are classified under integrin (receptor) family, immunoglobulin superfamily, selectins and cadherins. Integrins are made up of α and β subunits and are responsible for cell-cell and cell-matrix interactions. Immunoglobulin superfamily consists of CD2, CD58, intercellular adhesion molecules (ICAMs), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PE-CAM-1), and MAdCAM-1. Selectins are a group of cell adhesion molecules that are expressed on the surface of endothelial cells, leucocytes and platelets and have three subfamilies: E-selectin, P-selectin and L-selectin. Cadherins are a superfamily of Ca^{++} dependent cell adhesion molecules which are important in cell-cell interactions [42].

Early studies revealed two distinct structures in cell adhesions: close contacts and focal contacts which are separated by 30 nm and 10–15 nm from the substrate, respectively, in fibroblasts [43]. In recent studies, these contacts are classified as focal complexes, focal adhesions and fibrillary adhesions [44]. Focal complexes are located at the edge of a lamellipodium and are constituted of paxillin, vinculin, and tyrosine-phosphorylated proteins. Focal adhesions are located at the cell periphery and constitute of α_5 integrin, paxillin, vinculin, α actinin, talin, focal adhesion kinase (FAK), and tyrosine-phosphorylated proteins. Fibrillary adhesions are located in the central regions of cells and are made of α_5 integrin and tensin [44].

3. Adhesion of cells to ECM and mechanotransduction

Interactions of cells with the ECM and the neighboring cells elicit responses that have an essential role in the regulation of the behavior and fate of the cell. ECM constitutes a physical and chemical microenvironment, a site for anchorage of cells, and guides cell migration during embryonic development and wound repair. Therefore, it plays a key role in tissue morphogenesis. The ECM also acts as a carrier for the transmission of environmental signals to cells influencing proliferation, differentiation, and apoptosis [45]. Cells must sense, respond, and adapt to their physical environments at every level (molecular, cellular, tissue,