Available online at www.sciencedirect.com
ScienceDirect
Advanced Drug Delivery Reviews 59 (2007) 1306–1318
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Micromechanical control of cell and tissue development: Implications for tissue engineering ☆

Kaustabh Ghosh, Donald E. Ingber *

KFRL 11.127, Vascular Biology Program, Departments of Pathology and Surgery, Children's Hospital and Harvard Medical School, Boston, MA 02115, USA

Received 28 June 2007; accepted 1 August 2007
Available online 16 August 2007

Abstract

Tissue engineering approaches for repair of diseased or lost organs will require the development of new biomaterials that guide cell behavior and seamlessly integrate with living tissues. Previous approaches to engineer artificial tissues have focused largely on optimization of scaffold polymer chemistry and selection of appropriate biochemical additives (*e.g.*, growth factors, adhesive ligands) to provide effective developmental control. However, recent work has shown that micromechanical forces and local variations of extracellular matrix (ECM) elasticity at the microscale regulate cell and tissue development both *in vitro* and *in vivo*. The micromechanical properties of the host tissue microenvironment also play a critical role in control of stem cell lineage switching. Here we discuss how new understanding of the fundamental role that mechanical forces play in tissue development might be leveraged to facilitate the development of new types of biomimetic materials for regenerative medicine, with a focus on the design of injectable materials that can target to injury sites, recruit stem cells and direct cellular self-assembly to regenerate functional tissues and organs *in situ*.

Keywords: Tissue engineering; Cell–ECM interface; Tissue development; Scaffold

Contents

1. Introduction

The field of tissue engineering emerged more than two decades ago to address the acute shortage of organ transplants. The initial vision for the field was to isolate living cells from relevant organs of patients or other human donors, to culture them in polymer scaffolds that substitute for the body's natural extracellular matrix (ECM), and then to re-implant the cell-

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Natural and Artificial Cellular Microenvironments for Soft Tissue Repair".

* Corresponding author. Tel.: +1 617 919 2223; fax: +1 617 730 0230.
E-mail address: donald.ingber@childrens.harvard.edu (D.E. Ingber).

0169-409X/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.addr.2007.08.014

laden scaffolds into the patient in an attempt to replace or restore damaged tissues [1]. Several engineered tissues have been developed, with various chemical and biological modifications made to meet the specific demands of biocompatibility and biodegradability, and a few (*e.g.*, Apligraf, Integra artificial skin products) have been approved for clinical use. However, very few, if any, of these approaches have produced the ideal tissue substitute. This article is based on the belief that one reason for this failure may be the lack of in-depth understanding of the complex, dynamic and reciprocal interactions that occur at the cell–ECM interface in the native tissue microenvironment.

Typically, artificial ECM constructs, which form the backbone of most engineered tissues, are developed using either naturally-occurring molecular polymers (*e.g.* collagen, fibrin, hyaluronan *etc.*) or synthetic polymers (PLGA, PEG, PVA *etc.*). The adhesivity of the synthetic polymer scaffolds can be enhanced by being derivatized with ECM-derived peptides or protein fragments [2]. The RGD (arg-gly-asp) tripeptide found in various ECM molecules (*e.g.*, fibronectin, fibrin, *etc.*) is the most commonly used adhesive ligand because most cells bind to ECM in an RGD-dependent manner [3]. However, other adhesive peptides, such as the YIGSR and IKVAV peptides of laminin and VPGIG sequence of elastin, also have been studied [4–6]. These ligands are covalently linked to polymer backbones via their hydroxyl-, carboxyl- or amino-termini [3,7,8] so that they can physically resist cell traction forces, and the resulting engineered scaffolds can effectively support cell adhesion, spreading, proliferation and ECM synthesis [7,9,10]. Bioactivity of these peptides also can be further enhanced by altering their chemical structure. For example, RGD can supports better cell attachment and spreading when it is flanked by additional amino acid residues or when its conformation is changed from linear to cyclic [11].

To closely mimic native tissue architecture, ECM scaffolds have been commonly engineered in different physical forms such as hydrogels, macro- or microporous foams, as well as woven and non-woven fabrics; the porous nature of these materials facilitates delivery of oxygen and nutrients, and supports vascular tissue ingrowth [12–15]. Various cross-linking schemes (*e.g.* physical gelation, Michael-type addition, photoactivated cross-linking) are employed to achieve structural and proteolytic stability prior to or during cell seeding [16–18]. To impart greater functionality to engineered tissues that must bear large physical loads, for example, due to compression (cartilage, bone) or hemodynamic stresses (blood vessels), cell-seeded ECM constructs are sometimes subjected to controlled, tissue-level mechanical loading regimens *ex vivo* to enhance natural ECM accumulation prior to implantation [19–23]. Cells within these mechanically-conditioned artificial tissues often display increased viability and ECM synthesis both *in vitro* and *in vivo*.

Despite these variations in adhesive ligands, polymer chemistry, three-dimensional (3D) form, and mechanical loading, none of these engineered cell-scaffold constructs have resulted in complete restoration of normal tissue function when implanted *in vivo*. This failure may be, in part, based on the fact that cells not only respond to biochemical stimuli and large-scale (tissue- and organ-level) forces, but also to micromechanical forces conveyed to them through their ECM adhesions [24–27].

Recent studies have revealed that variations of ECM mechanics alter cell shape, intracellular biochemistry and gene expression based on their ability to resist cell traction forces, and thereby govern whether the cells will grow, die, move, contract or differentiate when stimulated with soluble stimuli [28–33]. Moreover, each tissue has its own characteristic mechanical properties that vary over the microscale, as well as a distinct chemical composition. These biochemical and mechanical signals interplay to guide tissue morphogenesis during embryological development, and to control stem cell lineage switching and wound healing in the adult [34–36]. In this chapter, we describe how micromechanical interactions between cells and ECM shape tissues during morphogenesis, and review the central role that cell shape, cytoskeletal tension and ECM mechanics play during tissue morphogenesis and developmental control. We also explore how this knowledge might inspire the development of novel inductive microenvironments for tissue growth and regeneration in the future.

2. Extracellular matrix as a physical determinant of tissue development

During embryogenesis, large cell populations self-organize into specific spatial patterns that give rise to different types of tissue and organs, and eventually to the whole organism. Although vital genetic and biochemical signals drive this developmental process, alone they are not sufficient to explain how 3D tissues are physically constructed so that they exhibit unique forms (*e.g.*, glandular epithelium, branching vascular networks) and mechanical load-bearing functions (tension-generating muscle, compression-resistant bone).

Cell-derived ECM molecules including laminin, fibronectin, proteoglycans and various collagen types regulate tissue development by self-assembling into physical anchoring scaffolds that guide multi-cellular organization. For example, self-assembly of collagen IV into a planar ‘chicken wire’-like mesh in the late blastocyst stage, combined with recruitment of laminin, proteoglycans and other ECM molecules to the surface of the network, results in formation of the first epithelial ECM, called ‘basement membrane’ [37,38]. Cells bind to this common anchoring platform through cell surface integrin receptors [39,40] and exert traction forces on their ECM adhesions [26,27,41–43]. The planar shape of the basement membrane therefore helps to establish the flattened form of the first epithelial sheets (ectoderm and endoderm). Importantly, whenever a new epithelium forms during later stages of development or during adult life, it is always accompanied by formation of a new basement membrane [44].

The basement membrane is not, however, simply a passive support structure; it also conveys signals through its adhesive contacts to neighboring adherent cells that control their shape, polarity, growth and function. For instance, the basement membrane enables these cells to develop cell–cell contacts and apical–basal polarity, which are essential for specialized