Chapter 10
Laser Printing Cells

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10.1 Introduction

Is there a laser-based tool that is used during surgery to regenerate tissue rather than cutting or cauterizing it? Not yet, but there are several innovative laser techniques under development that “print” cells and biomolecules layer-by-layer into organized three dimensional shapes that mimic the natural structure of living tissue. Akin to a biological mason that lays cells, extracellular matrix, and growth factors rather than bricks and mortar, these laser technologies are competing with ink jet, micropen, and electrospray methods to find a niche in tissue engineering applications. Some have gone as far to say that these printers could eventually be used to form pseudo-organs as an alternative to the current donor system, referring to this process as “organ printing” [1, 2].

The goal of tissue engineering research is to create in vitro 3D cell constructs, that once placed in an in vivo environment, differentiate to resemble natural systems as closely as possible (i.e., function, morphology, physical characteristics, etc.) [2-5]. Using cell printing to create heterogeneous 3D shapes with organized cell structure is an alternative to traditional tissue engineering approaches that involve homogeneous seeding of cells into porous three-dimensional scaffolding. Traditional scaffolds are made from highly sophisticated materials, usually biodegradable and/or biocompatible polymers, ceramics, or hydrogels [6-8]. In nearly all tissue engineering experiments, when cells meet scaffolding (seeding), the result is non-directed, homogenous coating of cells on the scaffold’s surface [2-5]. Several cell types can be simultaneously seeded onto scaffolding, but there exists no way to place different cell types in different areas of the scaffolding, much less on a length scale comparable to the heterogeneity found in natural tissue (10–100 μm). Growth factors and extracellular matrices can also be incorporated into scaffolding, even released with different time profiles [9-16], but again, the spatial distribution of these molecules is nearly always uniform across the entire scaffold. Certain cell types used in tissue engineering experiments can utilize natural (from the body) or artificial (from the scaffold) signals to initiate differentiation (angiogenesis, neurogenesis, etc.), but a scaffold with built-in blood vessel-like structures or neural networks with the proper channel size, surrounding cell types, and biomolecules have not been realized. These
are some of the challenges that could be addressed by laser printing cells into scaffolding rather than adding cells through random seeding.

There are two general approaches that use cell printing to create three-dimensional scaffolds. The first can be referred to as “structural” cell printing and requires that the same tool print the scaffolding, cells, and biomolecules simultaneously or sequentially (Fig. 10.1a). This category of cell printing is most relevant to ink jet cell printers and has been popularized by several recent news outlets and research articles under the term “organ printing” [2, 17]. There are several stringent and sometimes unattainable requirements for inks that are to be used in structural cell printing, as they not only need to support living cells but also polymerize or gel into a structurally sound, contiguous 3D shape post-printing. The second approach is more relevant to laser-based cell printing and can be referred to as “conformal” cell printing. Conformal cell printing is a hybrid approach that prints high resolution patterns of cells and biomolecules on top of thin layers of prefabricated scaffolding (Fig. 10.1b). Both approaches are similar in that the finished printed cell construct would be fabricated from the bottom up (i.e., layer-by-layer or cell-by-cell) and has heterogeneous cell and biomolecular structure in three dimensions, as required to mimic natural tissue or organs.

Conformal cell printing is a hybrid cell printing approach that builds upon previously refined scaffold technology. Conformal cell printing would potentially be able to utilize many more types of materials than “structural” printing because the pre- and post-processing requirements of the scaffolding material itself would not need to be conducive to printing (prefabricated layers of scaffolding). It would therefore be possible to use highly sophisticated scaffolds with nano-scale structure or biological/chemical modifications [9–16]. Conformal cell printing also may have the advantage of higher resolution than structural printing, at least initially, based on published reports of laser-based cell printers that have achieved single cell resolution [18, 19]. Conversely, these approaches add complexity by requiring more than just a cell printer in the experimental design (i.e., automated scaffold slicer and handler) and also face possible problems with overall structural integrity. However,

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**Fig. 10.1** Schematic representation of: (a) “structural” cell printing where both scaffold and cells are printed simultaneously or serially, and (b) “conformal” cell printing where cells alone are printed onto thin layers of prefabricated scaffolding. Laser-based cell printing was the first technique to demonstrate “conformal” printing.