2 Microfabrication of Cell-Laden Hydrogels for Engineering Mineralized and Load Bearing Tissues

Chia-Cheng Li, Mahshid Kharaziha, Christine Min, Richard Maas, and Mehdi Nikkhah

Abstract

Microengineering technologies and advanced biomaterials have extensive applications in the field of regenerative medicine. In this chapter, we review the integration of microfabrication techniques and hydrogel-based biomaterials in the field of dental, bone, and cartilage tissue engineering. We primarily discuss the major features that make hydrogels attractive candidates to mimic extracellular matrix (ECM), and we consider the benefits of three-dimensional (3D) culture systems for tissue engineering applications. We then focus on the fundamental principles of microfabrication techniques including photolithography, soft lithography and bioprinting approaches. Lastly, we summarize recent research on microengineering cell-laden hydrogel constructs for dental, bone and cartilage regeneration, and discuss future applications of microfabrication techniques for load-bearing tissue engineering.

Keywords

Load-bearing tissue engineering • Dentistry • Bone • Cartilage • Hydrogel • Microfabrication • Photolithography • Soft lithography • Bioprinting

C.-C. Li
Division of Genetics, Department of Medicine, Brigham and Women's Hospital, New Research Building, 458H, 77 Avenue Louis Pastuer, Boston, MA, USA

Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA, USA

M. Kharaziha
Department of Materials Engineering, Isfahan University of Technology, Isfahan, Iran

C. Min
Department of Oral Medicine, Infection, and Immunity, Harvard School of Dental Medicine, Boston, MA, USA

R. Maas, Ph.D. (✉)
Division of Genetics, Department of Medicine, Brigham and Women's Hospital, New Research Building, 458H, 77 Avenue Louis Pastuer, Boston, MA, USA
e-mail: maas@genetics.med.harvard.edu

M. Nikkhah, Ph.D. (✉)
Harrington Department of Biomedical Engineering, School of Biological and Health Systems Engineering, Arizona State University, ECG 334A, 501 E. Tyler Mall, Tempe, AZ 85287, USA
e-mail: mnikkhah@asu.edu

L.E. Bertassoni, P.G. Coelho (eds.), *Engineering Mineralized and Load Bearing Tissues*, Advances in Experimental Medicine and Biology 881, DOI 10.1007/978-3-319-22345-2_2

Abbreviations

2D	Two dimensional
3D	Three dimensional
BMP	Bone morphogenetic protein
μCP	Microcontact printing
DPSC	Dental pulp stem cell
ECM	Extracellular matrix
GelMA	Gelatin methacrylate
HA	Hydroxyapatite
MAPLE DW	Matrix assisted pulsed laser evaporation direct write
MSC	Mesenchymal stem cell
PCL	Poly-ε-caprolactone
PDL	Periodontal ligament
PDMS	Polydimethylsiloxane
PD-PEGDA	Photodegradable PEG diacrylate
PEG	Polyethylene glycol
PGA	Polyglycolic acid
PLGA	Poly-L-lactate-co-glycolic acid
PVA	Poly(vinyl-alcohol)
RGD	Arg-Gly-Asp
SCAP	Stem cells from apical papilla
SHED	Stem cells from human exfoliated deciduous teeth

2.1 Introduction

Load-bearing tissues, namely bone, cartilage and teeth, serve various physiological functions, including mechanical support, protection, as well as ion homeostasis (Gotfredsen and Walls 2007; Confavreux et al. 2011; Chen et al. 2013). Conditions such as trauma, infection or neoplasms impair the structures and functions of these tissues, and in turn significantly impact the life quality of patients (Gotfredsen and Walls 2007; Confavreux et al. 2011; Marcenes et al. 2013; Jackson et al. 2001). Medical treatments currently available for bone and cartilage reconstruction include grafts or artificial prostheses in addition to stable fixation (Finkemeier 2002). For tooth loss, dental implants or artificial crowns are the major treatment options (Sunnegardh-Gronberg et al. 2012). However, secondary infection, compromised biocompatibility, and the limited durability and accessibility of grafting materials and artificial prostheses remain major concerns (Finkemeier 2002; Puppi et al. 2010). To overcome these limitations, novel approaches that integrate stem cells and tissue engineering may provide valuable treatment alternatives for the regeneration of load-bearing tissues (Langer and Vacanti 1993; Cortesini 2005).

Tissue engineering is an interdisciplinary field that integrates biological sciences and bioengineering techniques to maintain, restore and enhance tissue or organ functions (Langer and Vacanti 1993). Tissue engineering approaches are mainly based on the use of isolated cell substitutes, acellular scaffolding biomaterials to initiate the regeneration process, or cell-laden biomaterials (Khademhosseini et al. 2006). While each approach possesses unique advantages, numerous challenges still exist such as the lack of renewable cell sources, a shortage of suitable biomaterials with enhanced mechanical, chemical, and biological properties, and an inability of in vivo revascularization (Khademhosseini et al. 2006; Langer and Vacanti 1999). The advancement of microfabrication techniques and biomaterial science in the past few years has paved the way to address some of the shortcomings of conventional tissue engineering (Khademhosseini et al. 2006). Microscale technologies were originally developed for fabricating semiconductor and microelectronic devices (Whitesides et al. 2001). Due to a wide range of length scale (i.e., 1–1,000 μm) and high resolution, microscale technologies provide a remarkable ability to facilitate the fabrication of miniaturized cell-laden constructs (Zorlutuna et al. 2012). Moreover, these technologies enable the precise control of the microenvironment, and organized vascularization for delivery of oxygenation and nutrients within engineered tissue constructs (Khademhosseini et al. 2006; Zorlutuna et al. 2012; Nikkhah et al. 2012a). In addition, the integration of microscale technologies with advanced biomaterials (e.g., hydrogels) promotes the development of high-throughput miniaturized assays to determine stem cell fate at single-cell level (Nikkhah et al. 2012a).

To date, microfabrication techniques have been applied for the development of load-bearing tissues (Petersen et al. 2002; Pelaez-Vargas et al. 2011). Using these technologies, it is possible to enhance cellular organization, tissue integration and interfacial strength (Charest et al. 2006; Gallant et al. 2007; Meredith et al. 2007; Kim et al. 2013a). In particular, the interfacial strength in cell-substrate interactions could be increased through deposition and adsorption of extracellular matrix (ECM) proteins on micro- and nanoscale patterned features (Kim et al. 2013b). Furthermore, these technologies facilitate the reciprocal cellular signaling, vascularization, and the delivery of growth factors for load-bearing tissue regeneration via precisely controlled spatial and temporal features of the cellular microenvironment (Kim et al. 2013a; Gray et al. 2003; Chung et al. 2007; Jager et al. 2008). This chapter outlines the applications of microscale technologies and hydrogel-based biomaterials for engineering load-bearing tissues. We first discuss the unique benefits of hydrogels in the development of engineered tissue constructs. Our discussion then focuses on fundamental microfabrication techniques, including photolithography, soft lithography and bioprinting. We finally highlight specific studies that are devoted toward the generation of cell-laden constructs for dental, bone and cartilage regeneration.

2.2 Hydrogels: Artificial Extracellular Matrices

The concept of tissue engineering stems from the ability of dissociated cells to recapitulate in vivo physiological functions under the appropriate settings (Kim and Mooney 1998). Since the ECM is important in tissue regeneration, an artificial ECM is normally used in tissue engineering to create a biomimetic microenvironment and to direct cell/tissue functions (Kim and Mooney 1998; Cohen et al. 1993). To date, numerous attempts have been made to develop synthetic or natural based biomaterials that closely resemble native ECM for tissue engineering applications (Kim and Mooney 1998; Tabata 2009). In this regard, hydrogels have attracted significant attention due to their suitable properties (Kim and Mooney 1998). Hydrogels are polymeric networks that are formed from hydrophilic polymers, and crosslinked to form insoluble gel matrices, which preserve a large amount of water (up to 99 %) (Peppas et al. 2006). The three-dimensional (3D) microenvironment of hydrogels circumvents some of the limitations of traditional two-dimensional (2D) cell culture systems (Petersen et al. 1992; Birgersdotter et al. 2005; Le Beyec et al. 2007). The biomimetic microenvironment within hydrogel constructs allows the diffusion of oxygen, nutrients and waste, as well as the transport of soluble factors (Slaughter et al. 2009). Due to their biocompatible nature, hydrogels have been widely used in regenerative medicine as an artificial ECM that provides cells with an initiating niche and support cell-cell and cell-matrix interactions (Slaughter et al. 2009).

Hydrogels can be fabricated from synthetic or naturally-derived materials (Peppas et al. 2006). Synthetic hydrogels (e.g., polyethylene glycol [PEG], polyglycolic acid [PGA], polyvinyl alcohol [PVA]) have the advantages of reproducible large-scale fabrication as well as tunable and consistent properties, but lack cell-recognizable motifs, such as Arg-Gly-Asp (RGD) (Kim and Mooney 1998). On the other hand, naturally-derived hydrogels (e.g., collagen, silk and hyaluronic acid) are attractive candidates for tissue engineering due to their biocompatibility and tunable biodegradability that support cell-matrix interactions (Peppas et al. 2006; Annabi et al. 2014). Compared to synthetic hydrogels, naturally-derived hydrogels offer a better optimized 3D microenvironment that promotes cell functions (e.g., attachment and proliferation) (Slaughter et al. 2009). However, the concerns of using naturally-derived hydrogels include low mechanical strength, batch-to-batch variance, and potential immunogenicity and contamination (Annabi et al. 2014). To further strengthen the mechanical properties of naturally-derived hydrogels, the incorporation of functional groups (e.g., acrylate) or other composites (e.g., synthetic hydrogels and nanoparticles) have been

studied (Ifkovits and Burdick 2007; Shin et al. 2013; DeKosky et al. 2010). Detailed descriptions on the properties and comparisons of various hydrogels are covered in previously published review articles (Peppas et al. 2006; Annabi et al. 2014).

Mechanical properties are key parameters when designing hydrogels for specific tissue engineering applications. In particular, the mechanical characteristics of hydrogel constructs, such as stiffness and ratio of stress/strain, have been shown to significantly influence cell behaviors (Huebsch et al. 2010; Baker and Chen 2012). Murine mesenchymal stem cells, for instance, differentiated toward an osteogenic fate in 3D RGD-modified hydrogels with stiffness similar to native osteoid matrix, which ranged from 11 to 30 kPa (Huebsch et al. 2010). Similarly, other cell types (e.g., fibroblasts in ligament and tendon) are capable of sensing stress and strain in the surrounding ECM, and respond accordingly by morphology, migration, proliferation and differentiation (Riehl et al. 2012). Beyond serving as scaffolds that support cell adhesion and promote cell-matrix interactions, hydrogels also regulate the spatial distribution of effector soluble molecules (e.g., morphogens, cytokines and growth factors) and gases through diffusive or convective transport as well as sequestration (Baker and Chen 2012). In this regard, techniques, such as microfabrication, have proven instrumental in adjusting the physical features (e.g., geometry and topography) of hydrogel constructs in order to support specific functionalities of multiple cell types within an organized tissue construct (Brock et al. 2003; Albrecht et al. 2006). As a result, the utility of cell-laden hydrogels in the field of regenerative medicine has seen a marked surge.

2.3 Microfabrication Techniques to Engineer Cell-Laden Hydrogels

One notable groundbreaking innovation in the field of tissue engineering is the use of microfabrication technology. So far, microfabrication techniques, including photolithography and soft lithography, have been widely applied for patterning or topographical guidance of cell-laden contructs (Andersson and van den Berg 2004). Tissue engineering has enormously benefited from microfabrication technology in terms of high flexibility, precise control in microenvironment design, efficient performance and cost-savings benefits due to the expediency for high-throughput and faster experiments (Andersson and van den Berg 2004). Below, we summarize the basic concepts and current applications of major microfabrication techniques.

2.3.1 Photolithography

Photolithography is a highly reliable microfabrication technique to manipulate features accurately at micro- and nano-scale (Liu Tsang et al. 2007; Shao and Fu 2014). In conventional photolithography, a photoresist is spin-coated uniformly on a flat substrate followed by exposure with UV light through a pre-fabricated photomask (Tabata 2009). UV light alters the chemical structure of a photoresist, further modifying its solubility in the developer solution and transferring the pattern of the photomask on the flat substrate (Borenstein et al. 2007). Through photolithography, it is possible to precisely pattern biomolecules or cells of interest on the substrate surface by etching or lift-off process in order to control the surface topographies (Andersson and van den Berg 2004; Liu Tsang et al. 2007). However, the major shortcoming of conventional photolithography is the high sensitivity of the procedure. Even the smallest dust particle can distort the spreading of photoresist molecules during the spinning process (Karp et al. 2006). Therefore, it is mandatory to carry out photolithography in a clean room (Karp et al. 2006) via relatively costly equipment (e.g. spin coater, mask aligner and wet benches) (Hwang et al. 2010).

On the other hand, hydrogel photolithography can be used on the bench-top to build 3D cell-laden constructs by the sequential patterning of photocrosslinkable hydrogels (Andersson and

van den Berg 2004; Liu Tsang et al. 2007). Compared to conventional photolithography, hydrogel photolithography is a fast, simple, and a low-cost technique. Photocrosslinkable hydrogels (e.g., gelatin methacrylate [GelMA], photodegradable PEG diacrylate [PD-PEGDA], methacrylated tropoelastin) can be used to manipulate cell behaviors (e.g., cell migration, cell proliferation and cell differentiation) and guide tissue organization (Khademhosseini et al. 2006; Moon et al. 2010a; Annabi et al. 2013). In a study by Nikkhah et al., endothelial cells-encapsulated GelMA constructs were precisely patterned with variable geometrical features using photolithography. The outcome of this study demonstrated that the cells rearranged toward the periphery of the constructs and formed highly organized cord-like structures that expressed endothelial cell markers, CD31 and VE-cadherin (Nikkhah et al. 2012b). This cord-like structure could act as a template during implantation to guide the formation of robust vessels integrated with the host tissue (Nikkhah et al. 2012b; Baranski et al. 2013).

2.3.2 Soft Lithography

Soft lithography (i.e., microcontact printing, microfluidic patterning) and replica molding techniques refer to a set of non-photolithographic approaches to develop 2D and 3D precisely ordered constructs with resolutions up to nanoscale (Whitesides et al. 2001; Yu and Ober 2003). In soft lithography, a prefabricated stamp or mold made of elastomeric polymers, such as polydimethylsiloxane (PDMS), is used to pattern biomolecules. On the other hand, replica molding techniques enable creating microscale features of heat-crosslinkable or photocrosslinkable hydrogels to control the distribution of the biomolecules or cells in a 3D microenvironment (McMillan et al. 1999; Selimovic et al. 2012; Occhetta et al. 2013).

Self-assembled monolayers, peptides and ECM can be efficiently patterned on various types of flat and curved surfaces using microcontact printing (μCP) (James et al. 1998). This technique facilitates the patterning of several molecules on a substrate using different stamps (Bernard et al. 2000), as well as a molecular gradient using stamps composed of arrays of high-resolution patterns (Crozatier et al. 2006). When using μCP, there are certain difficulties for patterning proteins on structurally soft substrates (e.g., hydrogels) (Damljanovic et al. 2005; Burnham et al. 2006; Rape et al. 2011), such as the stability of the biomolecules (Hynd et al. 2007). Therefore, a modified μCP process called "soft protein lithography" has been developed for patterning applications on hydrogel based surfaces (Polio et al. 2012; Turunen et al. 2013).

Microfluidic patterning refers to another set of soft lithography techniques, through which patterns can be created at desired locations of a substrate by restricting the flow within the microchannels formed by contacting a PDMS stamp on the substrate (Vanapalli et al. 2009). This technique was originally developed using capillary flow, but was further extended to pattern proteins and cells on larger channels (e.g., 100 μm) based on pressure-assisted flows. In this approach, using multi-layer PDMS stamps, it is possible to indirectly pattern different cell types at desired locations on a substrate (Chiu et al. 2000) or to generate heterogeneous multi-layer tissue constructs (Bernard et al. 2000; Vanapalli et al. 2009; Kenis et al. 1999; Jeon et al. 2000).

2.3.3 Bioprinting

Bioprinting has been utilized as a powerful tool to develop microscale engineered tissue constructs (Mironov et al. 2008; Moon et al. 2010b; Xu et al. 2010). Although bioprinting falls under the category of conventional microfabrication, the application of this technology to pattern biomolecules and cells holds unique benefits (Mironov et al. 2008). The major advantages of bioprinting include a fast and automated fabrication process and the development of 3D multi-layered constructs comprised of co-cultures of different cell types on a single substrate (Moon et al. 2010b; Mironov et al. 2003). Various types of bioprinting systems, such as inkjet-based

printing (Nakamura et al. 2005), laser printing (Barron et al. 2004; Nahmias et al. 2005), acoustic cell encapsulation (Demirci and Montesano 2007a) and valve-based printing (Demirci and Montesano 2007b; Song et al. 2010), have been used so far for tissue engineering applications. We refer readers to Chap. 1 of this book for more detail (Tasoglu and Demirci 2013).

2.4 Applications of Microfabrication Technology in Regenerative Dentistry

Teeth are highly mineralized organs used for various purposes, including mastication, phonetics and esthetics (Volponi et al. 2010). Although the morphology of teeth varies by species and location within the oral cavity, there is only slight variation in the composition of teeth, which consists of enamel, dentin, pulp, cementum, and periodontal ligament (PDL) (Yen and Sharpe 2008; Rodriguez-Lozano et al. 2012). Tooth loss due to periodontal disease, caries, trauma, or genetic predisposition remains a global health issue, and can significantly affect quality of life (Marcenes et al. 2013). Current treatment options for missing teeth are prosthetic replacements, such as crowns, bridges, dentures, and dental implants. A potentially attractive strategy for tooth replacement is tooth regeneration through the integration of biomimetic scaffolds, stem cells, cocktails of growth factors and micro- or nano-engineering technologies. It has been previously shown that extracted tooth buds from mouse embryos fully developed with correct orientation, size and morphology after transplanting into the diastema region of adult mice, suggesting that adult oral cavity provides a suitable environment for tooth regeneration (Ohazama et al. 2004). Furthermore, multiple cell types with odontogenic potency, such as dental pulp stem cells (DPSCs) (Gronthos et al. 2000) and stem cells from human exfoliated deciduous teeth (SHED) (Miura et al. 2003), have been identified as potential cell sources for tooth regeneration. These findings thus shed light on potential routes for the creation of bioengineered teeth to replace missing teeth in adults, through various combinations of embryonic tooth primordia and cultured progenitor cells (Nakao et al. 2007). Microfabrication may be particularly beneficial for regenerating the highly organized tissues of the tooth and periodontium since the microenvironment can be precisely controlled. In this section, we outline current accomplishments, challenges, and potential applications of microfabrication techniques in regenerative dentistry.

2.4.1 Regeneration of a Bioengineered Tooth

Odontogenesis is a strictly controlled developmental process that involves epithelial-mesenchymal interactions (O'Connell et al. 2012). To generate a whole tooth with its complex and mineralized load-bearing structures, a precisely-designed scaffold that can guide cell assembly and tissue organization is critical (Hacking and Khademhosseini 2009). With the aid of microfabrication, cell-laden hydrogel constructs can be prepared and spatially arranged with customized functional and architectural features (Khademhosseini et al. 2006). The scaffold-based approach typically involves harvesting, expanding, and differentiating the cells in vitro, seeding them onto pre-fabricated scaffolds, and subsequently implanting them in vivo (Fig. 2.1a) (Yen and Sharpe 2008; Yu et al. 2008).

To date, numerous biomaterials have been used in tooth regeneration studies, such as PGA (Duailibi et al. 2008), poly-L-lactate-co-glycolic acid (PLGA) (Duailibi et al. 2008), and collagen sponges (Fig. 2.1b) (Sumita et al. 2006). In one scaffold-based approach, a bioengineered tooth was generated by recombining and seeding dissociated epithelial cells and mesenchymal cells from an isolated embryonic tooth germ into a collagen gel droplet, and essentially reproducing the reciprocal epithelial-mesenchymal interactions in early odontogenesis (Nakao et al. 2007). To achieve the optimal size and morphology of teeth, a pre-fabricated scaffold that anatomically reflects the natural shape and size of a tooth has also been explored. Kim et al., in particular, used a 3D bioprinting technique to create a life-sized

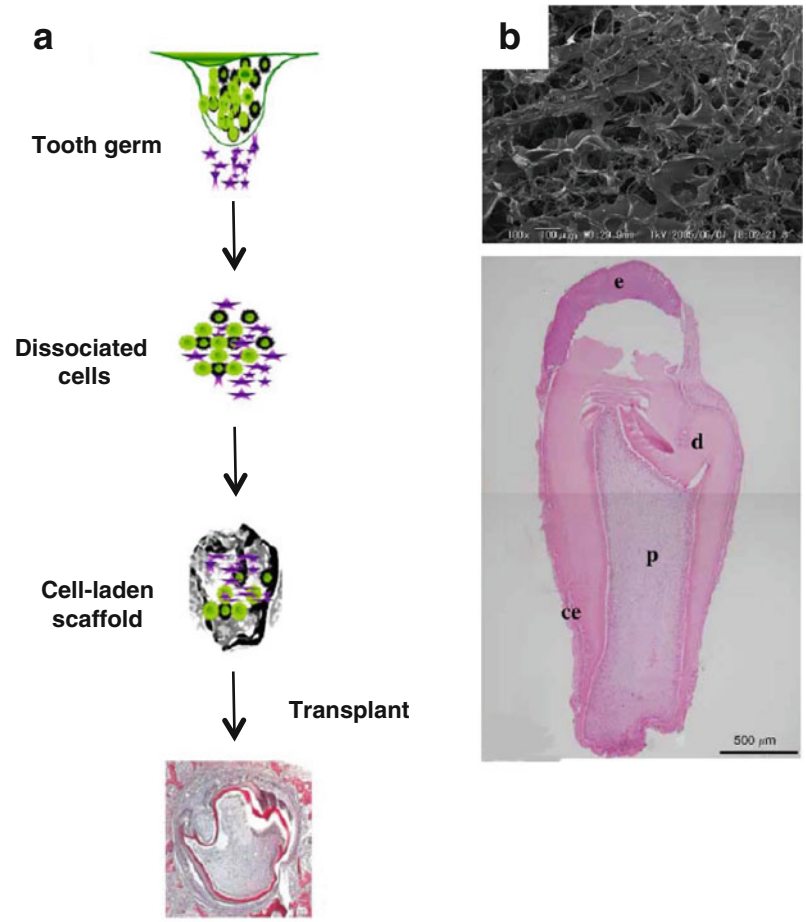

Fig. 2.1 Application of microfabrication technology to regenerative dentistry. (**a**) Schematic diagram of a scaffold-based approach, typically involving harvest of epithelial cells and mesenchymal cells from an embryonic tooth germ, followed by dissociation, recombination, and seeding onto a pre-fabricated tooth-shaped scaffold. Subsequently, the cell-laden construct is transplanted in vivo to generate a bioengineered tooth (Yen and Sharpe 2008) (Adapted from Yen and Sharpe with permission from **Cell and Tissue Research**. Copyright © 2007 Springer). (**b**) Application of microfabrication and novel biomaterials to generate a bioengineered tooth. *Upper panel*: Scanning electronic microscopy of the collagen sponge scaffold (Sumita et al. 2006). *Lower panel*: A bioengineered tooth that imitates anatomic tooth architecture formed 25-week post-transplantation, revealing enamel (*e*), dentin (*d*), cementum (*ce*) and pulp (*p*) (Sumita et al. 2006) (Adapted from Sumita et al. with permission from **Biomaterials**. Copyright © 2006 Elsevier)

tooth scaffold made of poly-ε-caprolactone (PCL) and hydroxyapatite (HA) with 200-μm-diameter interconnecting microchannels (Kim et al. 2010a). Moreover, the infusion of stromal-derived factor-1 (SDF1) and bone morphogenetic protein 7 (BMP7) into the microchannels of the scaffold was shown to recruit significantly more progenitor cells. Taken together, these findings demonstrate the potential of using a scaffold-based approach in regenerative dentistry (Kim et al. 2010b).

It is also important to consider the disadvantages of the scaffold-based approach for tooth regeneration, such as interrupted cell-matrix interactions, compromised biocompatibility, and poor preservation of growth factors within pre-fabricated scaffolds (Yu et al. 2008). However, these issues can be addressed by introducing alternative materials and modes of delivery. Cell-matrix interactions and biocompatibility, for instance, can be improved by using naturally-derived hydrogels, such as collagen and gelatin

(Slaughter et al. 2009). Furthermore, the microencapsulation or binding of critical growth factors to pre-fabricated scaffolds can prevent the unwanted diffusion of ligands (Carrasquillo et al. 2003; Lin et al. 2008), and this technique can potentially be applied for tooth regeneration.

2.4.2 Regeneration of Dental Pulpal Tissues

Regenerative endodontics aims to regenerate the dental pulp, which consists of vital neurovascular tissues. The integration of stem cells, scaffolds, and growth factors provides a promising avenue for revascularization and pulp tissue regeneration (Murray et al. 2007). In a recent study, DPSCs and stem cells from the apical papilla (SCAP) were seeded on a PLGA scaffold, inserted into the root canal spaces of root fragments, which were then implanted subcutaneously into immunocompromised mice. Three to four months after implantation, histological analysis showed pulp-like tissue and vascularization within the root canal spaces, as well as a continuous layer of dentin-like calcified deposition along the canal wall (Huang et al. 2010). While the exogenous application of stem cells is a commonly studied approach, one study suggested that an exogenous source may not be a critical component in regenerative endodontics (Volponi et al. 2010), and that proper vascularization may be sufficient to home progenitor cells into an empty canal for pulp regeneration. Microfabrication techniques have already been used to create vascular networks, and could potentially be used to enhance revascularization of the dental pulp in an organized and efficient manner (Nikkhah et al. 2012b; Morgan et al. 2013). There are, however, no major studies thus far that explore the use of microfabrication techniques for tooth revascularization.

2.4.3 Regeneration of Periodontium

Teeth are supported and anchored by the periodontium, which consists of cementum, periodontal ligament (PDL), gingiva, and alveolar bone. Since tooth loss occurs when these supporting structures are impaired by inflammatory conditions, such as severe periodontal disease, the restoration of these tissues is crucial. While more can be done, microfabrication has already demonstrated useful benefits in various studies of periodontal regeneration. Soft lithography, for instance, has been used to create modified surfaces that encourage periodontium regeneration. Pelaez-Vargas et al. demonstrated that micropatterned silica coatings on dental implant surfaces were biocompatible and notably capable of guiding the adhesion, spreading, and propagation of osteoblast-like cells for periodontal tissues regeneration (Pelaez-Vargas et al. 2011). 3D bioprinting has also been used to design a fiber-based scaffold to facilitate the formation of bone-ligament complexes that mimic the natural anatomy of the periodontium (Park et al. 2014; Ivanovski et al. 2014; Lee et al. 2014). With proper geometrical control, PDL fibers were regenerated in their proper orientation, and anchored in a cementum-like layer on the root surface. A multiphasic scaffold is another approach for regenerating the different components of the periodontium in a cohesive structure, and has only recently been considered (Ivanovski et al. 2014). In one study, 3D bioprinting was used to construct three continuous yet distinct phases: 100-μm microchannels with recombinant human amelogenin for the cementum/dentin interface, 600-μm microchannels with connective tissue growth factor for the PDL, and 300-μm microchannels with bone morphogenetic protein 2 for alveolar bone. The sizes of the microchannels for each phase were specifically chosen based on previous studies in fibro-osseous tissues regeneration. After in vivo implantation of the scaffold, PDL-like collagen fibers were seen inserted into bone-like and cementum-like tissues (Lee et al. 2014). The findings from in vitro and in vivo studies using multiphasic scaffolds, although promising, should be investigated further before large animal and human clinical trials.

Tooth and periodontium possess highly organized and complex structures. In this regard,

microengineering can be particularly useful for creating precisely designed platforms for dental tissue regeneration. Although significant progress has been made thus far in regenerative dentistry, more studies are warranted to eventually offer tooth and periodontium regeneration as a treatment option in a dental practice.

2.5 Applications of Microfabrication Technology in Bone Regeneration

Bone, which contributes to mechanical support and protection of the organism, is a complex mineralized organ containing collagenous fibrous matrix and nanocrystals of carbonated apatite (Weiner and Wagner 1998; Nguyen et al. 2012). In addition, bone plays critical biological roles in our bodies, such as ion homeostasis and hematopoiesis (Confavreux et al. 2011). Current medical management for severe bone damage consists of bone grafts (autografts, allografts, or xenografts) (Finkemeier 2002); however, several limitations exist due to the limited accessibility of graft materials, the morbidity of the donor sites, and potential for transmission of infectious pathogens (Simonds et al. 1992; Dimitriou et al. 2011). To eliminate these complications and to improve clinical outcome, novel biocompatible materials have been investigated for bone tissue engineering (Baler et al. 2014). These materials include: collagen (Geiger et al. 2003), calcium phosphate (Bucholz et al. 1989), ceramics and cements (Dorozhkin 2010), bioglasses (Bohner 2000), bioactive glass ceramics (Kinnunen et al. 2000), and a hybrid of PCL and nanocrystalline silicon-substituted hydroxyapatite (nano-SiHA) (Meseguer-Olmo et al. 2013). Studies have shown that SiHA possesses great bioactive behavior for bone formation (Porter et al. 2003), and that the addition of nanocrystalline ceramic particles can further enhance its biomineralization (Meseguer-Olmo et al. 2013). These nanocrystalline ceramic particles exhibit higher surface areas, and therefore have an enhanced dissolution rate and reactivity in contact with the surrounding tissue fluids (Meseguer-Olmo et al. 2013). In addition to the chemical compositions of the scaffolds, overall architecture of the constructs (e.g., density, pore shape, and pore size) and osteo-inductive biomolecules (e.g., BMP family members) rank among the other important qualities that encourage bone regeneration (Torricelli et al. 1999; Hutmacher 2000).

In addition to biocompatibility, biodegradability and accessibility, an ideal biomaterial for bone tissue engineering should meet other criteria, such as low viscosity for bioinjection and micromolding, and a capacity for incorporating cells or growth factors (Nguyen et al. 2012; Nguyen and Lee 2010). Injectable hydrogels, such as calcium alginate containing nano-HA (Tan et al. 2009) and nano-HA/PEG-PCL-PEG hydrogel nanocomposites (Fu et al. 2009), possess tunable injectability, degradability and setting time, and demonstrate in situ gelation activity. As a result, these biomaterials have been fabricated for bone tissue engineering, and several strategies have been employed to further enhance the calcification and mechanical strength of cell-laden hydrogel constructs. The incorporation of inorganic phases (e.g., calcium phosphates and bioglasses) into hydrogels is a common method to provide nucleation sites and induce physiological biomineralization (Kamitakahara et al. 2008; Gkioni et al. 2010; Rea et al. 2004). Use of a polymeric hydrogel backbone with anionic functional groups (e.g. $PO4^{3-}$, -COOH, and -OH groups), as well as incorporation of growth factors and osteoblast-like cells have also been suggested options to induce mineralization (Gkioni et al. 2010). Moreover, the degradation of hydrogel-based biomaterials allows for the replacement with newly-formed bone and for integration with surrounding native bone, thus increasing overall mechanical stability (Rezwan et al. 2006).

Microfabrication techniques have been utilized to introduce physiochemical cues within the 3D microenvironment (e.g., size, shape, porosity, stiffness, roughness and topography), and to influence the behavior of mesenchymal stem cells (MSCs) (Jiang et al. 2005). In recent studies, using photolithography, photoreactive PVA was

grafted on the polystyrene surfaces to construct micropatterns and provide a biocompatible platform for the long-term culture of MSCs. Bone marrow-derived MSCs were then cultured on these precisely-defined micropatterned PVA surfaces to investigate the effects of surface charge, cell spreading, seeding density and cell-cell interactions on MSC fate determination, including adipogenic, chondrogenic and osteogenic differentiation (Fig. 2.2a) (Wang et al. 2013; Lu et al. 2009; Song et al. 2011; Nedjari et al. 2014). In addition, micropatterning was utilized for studies in a single-cell level to reduce the heterogeneity of MSCs (Chen 2014). The findings from this study suggested that minimal cell-cell interactions, large cell spreading area, and increased contractility favored the osteogenic differentiation of MSCs (Wang et al. 2013; Chen 2014). μCP of biologically relevant ligands within cell-laden hydrogel constructs is another promising approach to achieve spatial control of ligand distribution (Park and Shuler 2003; Corum et al. 2011). The flexibility of pattern designs (e.g., shape and size) allows the micropatterned 3D co-cultures of cells, further facilitating cell proliferation and differentiation (Torisawa et al. 2010). μCP has also been applied to generate the micropatterns of bioactive glass nanoparticles on chitosan membranes, thereby regulating ionic release from these bioactive glass nanoparticles, maintaining the local pH value within the microenvironment, and enhancing biomineralization (Luz et al. 2012).

In addition, 3D bioprinting techniques have been demonstrated to develop cell-laden scaffolds that exhibit anatomically-shaped architecture, porosity and thickness for bone regeneration (Fig. 2.2b) (Meseguer-Olmo et al. 2013; Fedorovich et al. 2007; Murphy and Atala 2014). To achieve zonal distribution of multiple cell types, bioengineers have injected cell-laden hydrogel constructs that are gelled in situ or photopolymerized in layers, recapitulating the structures of native bone tissue (Fedorovich et al. 2007). Matrix assisted pulsed laser evaporation direct write (MAPLE DW) has been utilized for direct writing biomaterials and cells (Fig. 2.2c) (Schiele et al. 2010; Doraiswamy et al. 2007). This technique provides high accuracy in terms of spatial resolution, and improves cell-material integration. Co-deposition of osteoblast-like cells (MG63 cells) and bioceramic scaffold materials using the MAPLE DW strategy demonstrated that MG63 cells retained the viability and the capacity for proliferation, indicating this effective strategy can potentially be employed in cell-laden scaffolds for bone tissue engineering (Doraiswamy et al. 2007). Bottom-up approaches applying assembly of PCL and starch–PCL microfabricated sheets with human bone marrow stem cells allowed precisely imprinting microvasculatures and micropores (Lima et al. 2014).

2.6 Applications of Microfabrication Technology in Cartilage Regeneration

Cartilage tissue creates a nearly frictionless surface for joints to move and slide freely, but it may experience undesirable excessive forces and trauma (Jackson et al. 2001; Kim et al. 2012). The degeneration of articular cartilage and the associated osteoarthritis are one of the most prevalent age-related chronic conditions in the United States, affecting approximately 80 % of people older than 75 years old (Jackson et al. 2001). Disability caused by cartilage damage is an economic burden to the health care system, with a direct medical cost of roughly $15 billion each year (Jackson et al. 2001). Due to the avascularity and low mitotic activity of cartilage, it has a particularly limited capacity for self-healing when damaged (Buckwalter and Mankin 1998). The mainstay of treatment to repair damaged cartilage is still surgical intervention, such as arthroscopic lavage/debridement, autologous chondrocyte implantation, and osteochondral grafting (Kim et al. 2012). These surgical options provide temporary symptom relief and improve joint functions, but fail to fully restore damaged cartilage tissue (Kim et al. 2012). To address this limitation, bioengineering-based alternatives have been proposed to create an appropriate microenvironment and to regenerate cartilage tissue through the incorporation of cells, biochemical factors, and biomaterials (Petersen et al. 2002).

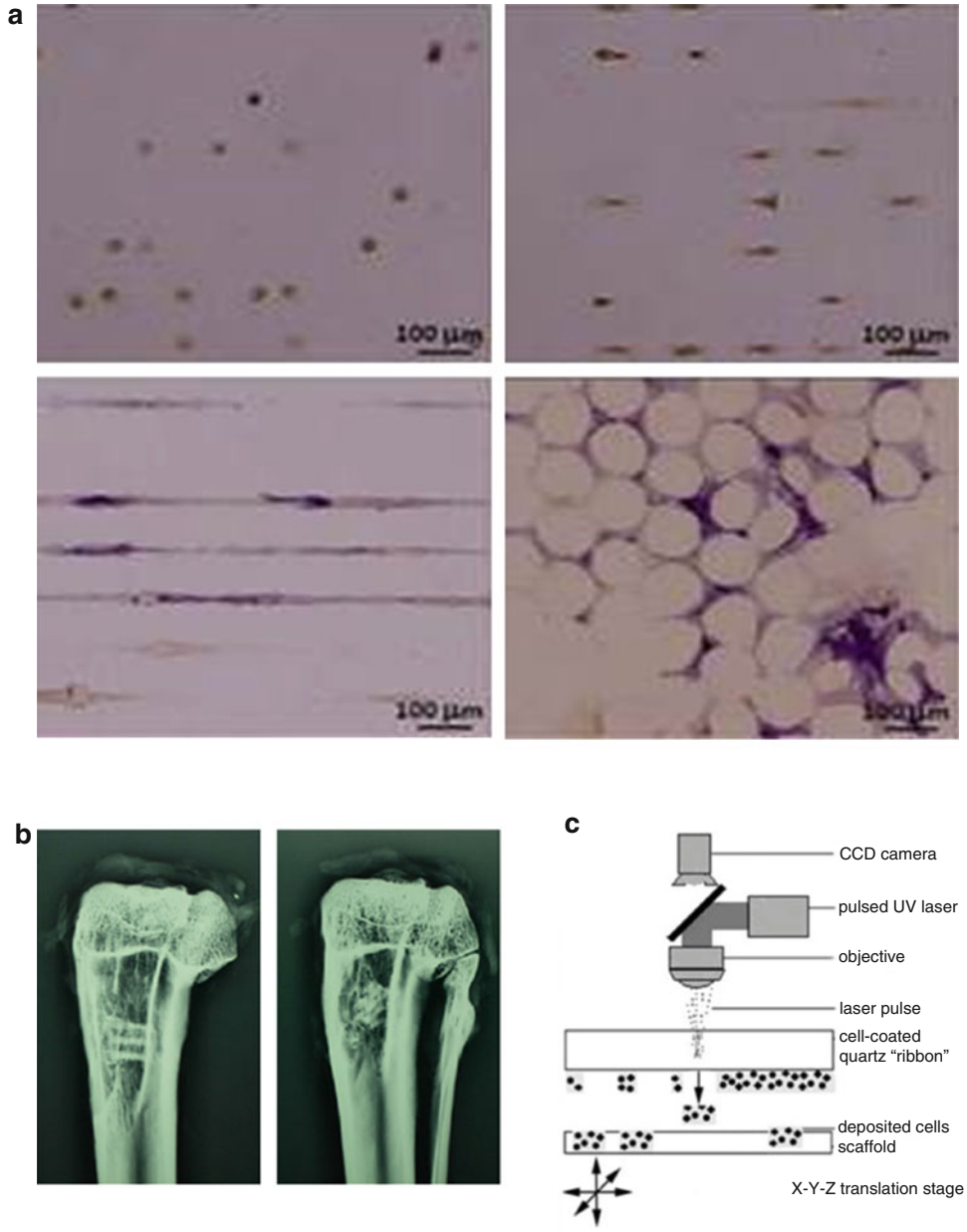

Fig. 2.2 Application of microfabrication technology to bone regeneration. (**a**) MSCs were cultured on the micropatterned surfaces for 2 weeks for osteo-induction. The Alkaline phosphatase (ALP) assay was used to investigate how different surface micropatterns influence osteogenic efficiency. *Purple* and *brown* colors indicate positive and negative staining for ALP, respectively (Wang et al. 2013) (Adapted from Wang et al. with permission from **Journal of Biomedical Materials Research**. Copyright © 2013 Wiley Periodicals, Inc). (**b**) Radiographic analyses of cell-laden construct implantation for bone regeneration. *Left panel*: At outset. *Right panel*: After 4 months (Meseguer-Olmo et al. 2013) (Adapted from Meseguer-Olmo et al. with permission from **Journal of Biomedical Materials Research**. Copyright © 2012 Wiley Periodicals, Inc). (**c**) Schematic diagram of the MAPLE DW process (Doraiswamy et al. 2007) (Adapted from Doraiswamy et al. with permission from **Journal of Biomedical Materials Research**. Copyright © 2006 Wiley Periodicals, Inc)

MSCs are an attractive cell source for cartilage regeneration due to their potential for chondrogenic differentiation under specific culture conditions (e.g., supplementation with transforming growth factor β [TGFβ]) (Pittenger et al. 1999; Diekman et al. 2010; Lai et al. 2013). Moreover, MSCs tend to commit to a chondrogenic fate when encapsulated in micropatterned constructs with high seeding density (Gao et al. 2010). In this regard, hydrogels can be applied as either cell-laden constructs to promote cartilage regeneration or cell-free implants to replace damaged cartilage (Spiller et al. 2011; Scaglione et al. 2014). Naturally-derived hydrogels, such as hyaluronic acid (Spiller et al. 2011) and elastin-like polypeptides (Mauck et al. 2000), are particularly appealing candidates due to their compositional similarity to cartilage ECM (Cushing and Anseth 2007). Furthermore, these naturally-derived hydrogels are able to enhance chondrogenic differentiation and proliferation (Spiller et al. 2011). However, these types of hydrogels are mechanically weak, and thus have limited use as cell-free implants for cartilage replacement (Spiller et al. 2011). Therefore, hybrid hydrogels consisting of natural and synthetic polymers have been suggested to strengthen the compressive and wear properties of constructs (Neves et al. 2011; Nguyen et al. 2011; Liao et al. 2013). In a study by Yasuda et al., double-network hydrogels of poly (2-acrylamido-2-methylpropane sulfonic acid) and poly(N,N-dimethyl acrylamide) were developed to imitate the collagen and glycosaminoglycan components of cartilage. These hydrogels exhibit low friction coefficients, and the compressive moduli are similar to articular cartilage (Yasuda et al. 2005). As the ECM of hyaline cartilage is a fiber-reinforced composite material, various kinds of composite hydrogels have been developed to mimic the mechanical and physical characteristics of native cartilage (Marijnissen et al. 2002; Chen et al. 2003; Ameer et al. 2002; Slivka et al. 2001). Slivka et al. developed PLGA hydrogels reinforced with polyglycolic acid fibers with mechanical properties in the range of native cartilage as a function of materials ratio (Slivka et al. 2001).

Since chondrocytes lose their phenotype and become fibroblast-like cells when cultured in vitro on traditional 2D cell culture substrates (Freed et al. 1999), it is important to control cell-cell and cell-ECM interactions, and to maintain the chondrogenic features of the cell-laden constructs (Petersen et al. 2002). With micropatterning techniques, it is possible to develop a well-defined substrate that can guide the chondrogenic differentiation of progenitor cells. Surface-patterned scaffolds that were prefabricated to support chondrogenesis demonstrated the capacity to promote adhesion, restrict spreading, maintain chondrocytic phenotypes and support the deposition of cartilage ECM (e.g., aggregan) (Petersen et al. 2002). In another study employing PEG hydrogels and photolithography, 2D microarrays of cell-adhesive circular domains ($\phi = 100$ μm) surrounded by PEG-coated cytophobic areas were constructed to promote the aggregation and spheroid formation of chondrocytes (Otsuka et al. 2012). This approach demonstrated its capacity to stimulate aggrecan production, and to maintain the chondrocytic spheroids for more than a month (Otsuka et al. 2012).

In addition to micropatterning techniques, 3D cell-laden biomimetic microengineered constructs can be used to imitate the architectural and mechanical characteristics of target organs or tissues (e.g., cartilage) (Klein et al. 2009). Articular cartilage exhibits zonal maturation with variations in cell phenotype, matrix organization, composition and mechanical properties along the depth of the cartilage plate. Multi-layered photocrosslinkable hydrogels can be used to recreate the biomimetic zonal maturation of articular cartilage (Nguyen et al. 2011; Sharma et al. 2007). Photodegradable PEG-based hydrogels were applied to encapsulate stem cells, and post-gelation control of the constructs was demonstrated to introduce dynamic temporo-spatial changes and to affect cell migration and chondrogenic differentiation (Kloxin et al. 2009). In another study by Xu et al., a hybrid bioprinting/electrospinning approach was utilized to develop layer-by-layer chondrocyte-laden fibrin/collagen hydrogel constructs for cartilage tissue engineering. Compared to the conventional bioprinting

method, the proposed hybrid approach enhanced mechanical properties of the constructs, maintained cell viability, and induced the deposition of cartilage ECM both in vitro and in vivo (Xu et al. 2013). Further refinement of this hybrid technique to produce oriented fibers is envisioned to guide chondrocyte growth and to construct functional cartilage tissues.

2.7 Conclusion and Future Perspectives

During the past decade, significant progress in microfabrication and biomaterial science has been made in developing complex biomimetic transplantable constructs that can guide cell growth and differentiation as well as tissue organization. However, challenges still remain, such as achieving the precise control of 3D cell-laden constructs, dynamic changes in microenvironment, and in the enhancement of revascularization. The development of improved scaffolds with customized physical characteristics is critical, and microfabrication with higher resolution is likely to prove important. Innovative and optimized microfabrication techniques are essential for enriching specific biological functions, such as cell seeding and vascularization, as well as for facilitating the natural healing process in vivo. Beyond advances in bioengineering, it is also attractive to incorporate biochemical cues within 3D cell-laden constructs. A thorough understanding of the underlying biological mechanisms for these load-bearing organs development is thus a necessary pre-requisite. The integration of biological insights and bioengineering technologies will help to significantly advance the field of regenerative medicine.

References

Albrecht DR, Underhill GH, Wassermann TB, Sah RL, Bhatia SN (2006) Probing the role of multicellular organization in three-dimensional microenvironments. Nat Methods 3:369–375

Ameer GA, Mahmood TA, Langer R (2002) A biodegradable composite scaffold for cell transplantation. J Orthop Res 20:16–19

Andersson H, van den Berg A (2004) Microfabrication and microfluidics for tissue engineering: state of the art and future opportunities. Lab Chip 4:98–103

Annabi N et al (2013) Highly elastic micropatterned hydrogel for engineering functional cardiac tissue. Adv Funct Mater 23:4950–4959

Annabi N et al (2014) 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. Adv Mater 26:85–124

Baker BM, Chen CS (2012) Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues. J Cell Sci 125:3015–3024

Baler K et al (2014) Advanced nanocomposites for bone regeneration. Biomater Sci 2:1355–1366

Baranski JD et al (2013) Geometric control of vascular networks to enhance engineered tissue integration and function. Proc Natl Acad Sci U S A 110:7586–7591

Barron JA, Ringeisen BR, Kim H, Spargo BJ, Chrisey DB (2004) Application of laser printing to mammalian cells. Thin Solid Films 453:383–387

Bernard A, Renault JP, Michel B, Bosshard HR, Delamarche E (2000) Microcontact printing of proteins. Adv Mater 12:1067–1070

Birgersdotter A, Sandberg R, Ernberg I (2005) Gene expression perturbation in vitro – a growing case for three-dimensional (3D) culture systems. Semin Cancer Biol 15:405–412

Bohner M (2000) Calcium orthophosphates in medicine: from ceramics to calcium phosphate cements. Injury 31(Suppl 4):37–47

Borenstein JT et al (2007) Microfabrication of three-dimensional engineered scaffolds. Tissue Eng 13:1837–1844

Brock A et al (2003) Geometric determinants of directional cell motility revealed using microcontact printing. Langmuir 19:1611–1617

Bucholz RW, Carlton A, Holmes R (1989) Interporous hydroxyapatite as a bone graft substitute in tibial plateau fractures. Clin Orthop Relat Res 240:53–62

Buckwalter JA, Mankin HJ (1998) Articular cartilage: degeneration and osteoarthritis, repair, regeneration, and transplantation. Instr Course Lect 47:487–504

Burnham MR, Turner JN, Szarowski D, Martin DL (2006) Biological functionalization and surface micropatterning of polyacrylamide hydrogels. Biomaterials 27:5883–5891

Carrasquillo KG et al (2003) Controlled delivery of the anti-VEGF aptamer EYE001 with poly(lactic-co-glycolic) acid microspheres. Invest Ophthalmol Vis Sci 44:290–299

Charest JL, Eliason MT, Garcia AJ, King WP (2006) Combined microscale mechanical topography and chemical patterns on polymer cell culture substrates. Biomaterials 27:2487–2494

Chen G (2014) Poly(vinyl alcohol)-micropatterned surfaces for manipulation of mesenchymal stem cell functions. Methods Cell Biol 119:17–33

Chen G et al (2003) The use of a novel PLGA fiber/collagen composite web as a scaffold for engineering of articular cartilage tissue with adjustable thickness. J Biomed Mater Res A 67:1170–1180

Chen C, Tambe DT, Deng L, Yang L (2013) Biomechanical properties and mechanobiology of the articular chondrocyte. Am J Physiol Cell Physiol 305:C1202–C1208

Chiu DT et al (2000) Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems. Proc Natl Acad Sci U S A 97:2408–2413

Chung BG, Kang L, Khademhosseini A (2007) Micro- and nanoscale technologies for tissue engineering and drug discovery applications. Expert Opin Drug Discov 2:1653–1668

Cohen S et al (1993) Design of synthetic polymeric structures for cell transplantation and tissue engineering. Clin Mater 13:3–10

Confavreux CB (2011) Bone: from a reservoir of minerals to a regulator of energy metabolism. Kidney Int Suppl 79:S14–S19

Cortesini R (2005) Stem cells, tissue engineering and organogenesis in transplantation. Transpl Immunol 15:81–89

Corum LE, Eichinger CD, Hsiao TW, Hlady V (2011) Using microcontact printing of fibrinogen to control surface-induced platelet adhesion and activation. Langmuir 27:8316–8322

Crozatier C, Berre ML, Chen Y (2006) Multi-colour micro-contact printing based on microfluidic network inking. Microelectron Eng 83:910–913

Cushing MC, Anseth KS (2007) Materials science. Hydrogel Cell Cult Sci 316:1133–1134

Damljanovic V, Lagerholm BC, Jacobson K (2005) Bulk and micropatterned conjugation of extracellular matrix proteins to characterized polyacrylamide substrates for cell mechanotransduction assays. Biotechniques 39:847–851

DeKosky BJ et al (2010) Hierarchically designed agarose and poly(ethylene glycol) interpenetrating network hydrogels for cartilage tissue engineering. Tissue Eng Part C Methods 16:1533–1542

Demirci U, Montesano G (2007a) Single cell epitaxy by acoustic picolitre droplets. Lab Chip 7:1139–1145

Demirci U, Montesano G (2007b) Cell encapsulating droplet vitrification. Lab Chip 7:1428–1433

Diekman BO, Rowland CR, Lennon DP, Caplan AI, Guilak F (2010) Chondrogenesis of adult stem cells from adipose tissue and bone marrow: induction by growth factors and cartilage-derived matrix. Tissue Eng Part A 16:523–533

Dimitriou R, Mataliotakis GI, Angoules AG, Kanakaris NK, Giannoudis PV (2011) Complications following autologous bone graft harvesting from the iliac crest and using the RIA: a systematic review. Injury 42(Suppl 2):S3–S15

Doraiswamy A et al (2007) Laser microfabrication of hydroxyapatite-osteoblast-like cell composites. J Biomed Mater Res A 80:635–643

Dorozhkin SV (2010) Bioceramics of calcium orthophosphates. Biomaterials 31:1465–1485

Duailibi SE et al (2008) Bioengineered dental tissues grown in the rat jaw. J Dent Res 87:745–750

Fedorovich NE et al (2007) Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. Tissue Eng 13:1905–1925

Finkemeier CG (2002) Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am 84-A:454–464

Freed LE, Martin I, Vunjak-Novakovic G (1999) Frontiers in tissue engineering. In vitro modulation of chondrogenesis. Clin Orthop Relat Res 367:S46–S58

Fu S et al (2009) Injectable biodegradable thermosensitive hydrogel composite for orthopedic tissue engineering. 1. Preparation and characterization of nanohydroxyapatite/poly(ethyleneglycol)-poly(epsilon-caprolactone)-poly(ethylene glycol) hydrogel nanocomposites. J Phys Chem B 113:16518–16525

Gallant ND, Charest JL, King WP, Garcia AJ (2007) Micro- and nano-patterned substrates to manipulate cell adhesion. J Nanosci Nanotechnol 7:803–807

Gao L, McBeath R, Chen CS (2010) Stem cell shape regulates a chondrogenic versus myogenic fate through Rac1 and N-cadherin. Stem Cells 28:564–572

Geiger M, Li RH, Friess W (2003) Collagen sponges for bone regeneration with rhBMP-2. Adv Drug Deliv Rev 55:1613–1629

Gkioni K, Leeuwenburgh SC, Douglas TE, Mikos AG, Jansen JA (2010) Mineralization of hydrogels for bone regeneration. Tissue Eng Part B Rev 16:577–585

Gotfredsen K, Walls AW (2007) What dentition assures oral function? Clin Oral Implants Res 18(Suppl 3):34–45

Gray DS, Tien J, Chen CS (2003) Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's modulus. J Biomed Mater Res A 66:605–614

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97:13625–13630

Hacking SA, Khademhosseini A (2009) Applications of microscale technologies for regenerative dentistry. J Dent Res 88:409–421

Huang GT et al (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 16:605–615

Huebsch N et al (2010) Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nat Mater 9:518–526

Hutmacher DW (2000) Scaffolds in tissue engineering bone and cartilage. Biomaterials 21:2529–2543

Hwang CM et al (2010) Benchtop fabrication of PDMS microstructures by an unconventional photolithographic method. Biofabrication 2:045001

Hynd MR, Frampton JP, Dowell-Mesfin N, Turner JN, Shain W (2007) Directed cell growth on protein-functionalized hydrogel surfaces. J Neurosci Methods 162:255–263

Ifkovits JL, Burdick JA (2007) Review: photopolymerizable and degradable biomaterials for tissue engineering applications. Tissue Eng 13:2369–2385

Ivanovski S, Vaquette C, Gronthos S, Hutmacher DW, Bartold PM (2014) Multiphasic scaffolds for periodontal tissue engineering. J Dent Res 93:1212–1221

Jackson DW, Simon TM, Aberman HM (2001) Symptomatic articular cartilage degeneration: the impact in the new millennium. Clin Orthop Relat Res 391:S14–S25

Jager M et al (2008) Osteoblast differentiation onto different biometals with an endoprosthetic surface topography in vitro. J Biomed Mater Res A 86:61–75

James C et al (1998) Patterned protein layers on solid substrates by thin stamp microcontact printing. Langmuir 14:741–744

Jeon NL et al (2000) Generation of solution and surface gradients using microfluidic systems. Langmuir 16:8311–8316

Jiang X, Bruzewicz DA, Wong AP, Piel M, Whitesides GM (2005) Directing cell migration with asymmetric micropatterns. Proc Natl Acad Sci U S A 102:975–978

Kamitakahara M, Ohtsuki C, Miyazaki T (2008) Review paper: behavior of ceramic biomaterials derived from tricalcium phosphate in physiological condition. J Biomater Appl 23:197–212

Karp JM et al (2006) A photolithographic method to create cellular micropatterns. Biomaterials 27:4755–4764

Kenis PJ, Ismagilov RF, Whitesides GM (1999) Microfabrication inside capillaries using multiphase laminar flow patterning. Science 285:83–85

Khademhosseini A, Langer R, Borenstein J, Vacanti JP (2006) Microscale technologies for tissue engineering and biology. Proc Natl Acad Sci U S A 103:2480–2487

Kim BS, Mooney DJ (1998) Development of biocompatible synthetic extracellular matrices for tissue engineering. Trends Biotechnol 16:224–230

Kim K, Lee CH, Kim BK, Mao JJ (2010a) Anatomically shaped tooth and periodontal regeneration by cell homing. J Dent Res 89:842–847

Kim JY et al (2010b) Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. Tissue Eng Part A 16:3023–3031

Kim K, Yoon DM, Mikos A, Kasper FK (2012) Harnessing cell-biomaterial interactions for osteochondral tissue regeneration. Adv Biochem Eng Biotechnol 126:67–104

Kim J et al (2013a) Designing nanotopographical density of extracellular matrix for controlled morphology and function of human mesenchymal stem cells. Sci Rep 3:3552

Kim HN et al (2013b) Nanotopography-guided tissue engineering and regenerative medicine. Adv Drug Deliv Rev 65:536–558

Kinnunen I, Aitasalo K, Pollonen M, Varpula M (2000) Reconstruction of orbital floor fractures using bioactive glass. J Craniomaxillofac Surg 28:229–234

Klein TJ, Malda J, Sah RL, Hutmacher DW (2009) Tissue engineering of articular cartilage with biomimetic zones. Tissue Eng Part B Rev 15:143–157

Kloxin AM, Kasko AM, Salinas CN, Anseth KS (2009) Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science 324:59–63

Lai JH, Kajiyama G, Smith RL, Maloney W, Yang F (2013) Stem cells catalyze cartilage formation by neonatal articular chondrocytes in 3D biomimetic hydrogels. Sci Rep 3:3553

Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920–926

Langer RS, Vacanti JP (1999) Tissue engineering: the challenges ahead. Sci Am 280:86–89

Le Beyec J et al (2007) Cell shape regulates global histone acetylation in human mammary epithelial cells. Exp Cell Res 313:3066–3075

Lee CH et al (2014) Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex. Tissue Eng Part A 20:1342–1351

Liao IC, Moutos FT, Estes BT, Zhao X, Guilak F (2013) Composite three-dimensional woven scaffolds with interpenetrating network hydrogels to create functional synthetic articular cartilage. Adv Funct Mater 23:5833–5839

Lima MJ et al (2014) Bottom-up approach to construct microfabricated multi-layer scaffolds for bone tissue engineering. Biomed Microdevices 16:69–78

Lin H et al (2008) The effect of crosslinking heparin to demineralized bone matrix on mechanical strength and specific binding to human bone morphogenetic protein-2. Biomaterials 29:1189–1197

Liu Tsang V et al (2007) Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. FASEB J 21:790–801

Lu H et al (2009) Effect of cell density on adipogenic differentiation of mesenchymal stem cells. Biochem Biophys Res Commun 381:322–327

Luz GM, Boesel L, del Campo A, Mano JF (2012) Micropatterning of bioactive glass nanoparticles on chitosan membranes for spatial controlled biomineralization. Langmuir 28:6970–6977

Marcenes W et al (2013) Global burden of oral conditions in 1990–2010: a systematic analysis. J Dent Res 92:592–597

Marijnissen WJ et al (2002) Alginate as a chondrocyte-delivery substance in combination with a non-woven scaffold for cartilage tissue engineering. Biomaterials 23:1511–1517

Mauck RL et al (2000) Functional tissue engineering of articular cartilage through dynamic loading of chondrocyte-seeded agarose gels. J Biomech Eng 122:252–260

McMillan RA, Caran KL, Apkarian RP, Conticello VP (1999) High-resolution topographic imaging of environmentally responsive, elastin-mimetic hydrogels. Macromolecules 32:9067–9070

Meredith DO, Eschbach L, Riehle MO, Curtis AS, Richards RG (2007) Microtopography of metal surfaces influence fibroblast growth by modifying cell shape, cytoskeleton, and adhesion. J Orthop Res 25:1523–1533

Meseguer-Olmo L et al (2013) In-vivo behavior of Si-hydroxyapatite/polycaprolactone/DMB scaffolds fabricated by 3D printing. J Biomed Mater Res A 101:2038–2048

Mironov V, Boland T, Trusk T, Forgacs G, Markwald RR (2003) Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol 21: 157–161

Mironov V, Kasyanov V, Drake C, Markwald RR (2008) Organ printing: promises and challenges. Regen Med 3:93–103

Miura M et al (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100:5807–5812

Moon JJ et al (2010a) Biomimetic hydrogels with pro-angiogenic properties. Biomaterials 31:3840–3847

Moon S et al (2010b) Layer by layer three-dimensional tissue epitaxy by cell-laden hydrogel droplets. Tissue Eng Part C Methods 16:157–166

Morgan JP et al (2013) Formation of microvascular networks in vitro. Nat Protoc 8:1820–1836

Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. Nat Biotechnol 32:773–785

Murray PE, Garcia-Godoy F, Hargreaves KM (2007) Regenerative endodontics: a review of current status and a call for action. J Endod 33:377–390

Nahmias Y, Schwartz RE, Verfaillie CM, Odde DJ (2005) Laser-guided direct writing for three-dimensional tissue engineering. Biotechnol Bioeng 92:129–136

Nakamura M et al (2005) Biocompatible inkjet printing technique for designed seeding of individual living cells. Tissue Eng 11:1658–1666

Nakao K et al (2007) The development of a bioengineered organ germ method. Nat Methods 4:227–230

Nedjari S et al (2014) Electrospun honeycomb as nests for controlled osteoblast spatial organization. Macromol Biosci 14:1580–1589

Neves SC et al (2011) Chitosan/poly(epsilon-caprolactone) blend scaffolds for cartilage repair. Biomaterials 32:1068–1079

Nguyen MK, Lee DS (2010) Injectable biodegradable hydrogels. Macromol Biosci 10:563–579

Nguyen LH, Kudva AK, Guckert NL, Linse KD, Roy K (2011) Unique biomaterial compositions direct bone marrow stem cells into specific chondrocytic phenotypes corresponding to the various zones of articular cartilage. Biomaterials 32:1327–1338

Nguyen LH et al (2012) Vascularized bone tissue engineering: approaches for potential improvement. Tissue Eng Part B Rev 18:363–382

Nikkhah M, Edalat F, Manoucheri S, Khademhosseini A (2012a) Engineering microscale topographies to control the cell-substrate interface. Biomaterials 33:5230–5246

Nikkhah M et al (2012b) Directed endothelial cell morphogenesis in micropatterned gelatin methacrylate hydrogels. Biomaterials 33:9009–9018

O'Connell DJ et al (2012) A Wnt-bmp feedback circuit controls intertissue signaling dynamics in tooth organogenesis. Sci Signal 5:ra4

Occhetta P et al (2013) Fabrication of 3D cell-laden hydrogel microstructures through photo-mold patterning. Biofabrication 5:035002

Ohazama A, Modino SA, Miletich I, Sharpe PT (2004) Stem-cell-based tissue engineering of murine teeth. J Dent Res 83:518–522

Otsuka H et al (2012) Chondrocyte spheroids on microfabricated PEG hydrogel surface and their noninvasive functional monitoring. Sci Technol Adv Mater 13:064217

Park TH, Shuler ML (2003) Integration of cell culture and microfabrication technology. Biotechnol Prog 19:243–253

Park CH et al (2014) Image-based, fiber guiding scaffolds: a platform for regenerating tissue interfaces. Tissue Eng Part C Methods 20:533–542

Pelaez-Vargas A et al (2011) Isotropic micropatterned silica coatings on zirconia induce guided cell growth for dental implants. Dent Mater 27:581–589

Peppas NA, Hilt JZ, Khademhosseini A, Langer R (2006) Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Adv Mater 18:1345–1360

Petersen OW, Ronnov-Jessen L, Howlett AR, Bissell MJ (1992) Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proc Natl Acad Sci U S A 89:9064–9068

Petersen EF, Spencer RG, McFarland EW (2002) Microengineering neocartilage scaffolds. Biotechnol Bioeng 78:801–804

Pittenger MF et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147

Polio SR, Rothenberg KE, Stamenovic D, Smith ML (2012) A micropatterning and image processing approach to simplify measurement of cellular traction forces. Acta Biomater 8:82–88

Porter AE, Patel N, Skepper JN, Best SM, Bonfield W (2003) Comparison of in vivo dissolution processes in hydroxyapatite and silicon-substituted hydroxyapatite bioceramics. Biomaterials 24:4609–4620

Puppi D, Chiellini F, Piras AM, Chiellini E (2010) Polymeric materials for bone and cartilage repair. Prog Polym Sci 35:403–440

Rape AD, Guo WH, Wang YL (2011) The regulation of traction force in relation to cell shape and focal adhesions. Biomaterials 32:2043–2051

Rea SM, Best SM, Bonfield W (2004) Bioactivity of ceramic-polymer composites with varied composition and surface topography. J Mater Sci Mater Med 15:997–1005

Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR (2006) Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 27:3413–3431

Riehl BD, Park JH, Kwon IK, Lim JY (2012) Mechanical stretching for tissue engineering: two-dimensional and three-dimensional constructs. Tissue Eng Part B Rev 18:288–300

Rodriguez-Lozano FJ et al (2012) Mesenchymal dental stem cells in regenerative dentistry. Med Oral Patol Oral Cir Bucal 17:e1062–e1067

Scaglione S et al (2014) A novel scaffold geometry for chondral applications: theoretical model and in vivo validation. Biotechnol Bioeng 111:2107–2119

Schiele NR et al (2010) Laser-based direct-write techniques for cell printing. Biofabrication 2:032001

Selimovic S, Oh J, Bae H, Dokmeci M, Khademhosseini A (2012) Microscale strategies for generating cell-encapsulating hydrogels. Polym (Basel) 4:1554

Shao Y, Fu J (2014) Integrated micro/nanoengineered functional biomaterials for cell mechanics and mechanobiology: a materials perspective. Adv Mater 26:1494–1533

Sharma B et al (2007) Designing zonal organization into tissue-engineered cartilage. Tissue Eng 13:405–414

Shin SR et al (2013) Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators. ACS Nano 7:2369–2380

Simonds RJ et al (1992) Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. N Engl J Med 326:726–732

Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA (2009) Hydrogels in regenerative medicine. Adv Mater 21:3307–3329

Slivka MA, Leatherbury NC, Kieswetter K, Niederauer GG (2001) Porous, resorbable, fiber-reinforced scaffolds tailored for articular cartilage repair. Tissue Eng 7:767–780

Song YS et al (2010) Vitrification and levitation of a liquid droplet on liquid nitrogen. Proc Natl Acad Sci U S A 107:4596–4600

Song W, Lu H, Kawazoe N, Chen G (2011) Gradient patterning and differentiation of mesenchymal stem cells on micropatterned polymer surface. J Bioact Compat Polym 26:242–256

Spiller KL, Maher SA, Lowman AM (2011) Hydrogels for the repair of articular cartilage defects. Tissue Eng Part B Rev 17:281–299

Sumita Y et al (2006) Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. Biomaterials 27:3238–3248

Sunnegardh-Gronberg K et al (2012) Treatment of adult patients with partial edentulism: a systematic review. Int J Prosthodont 25:568–581

Tabata Y (2009) Biomaterial technology for tissue engineering applications. J R Soc Interface 6(Suppl 3):S311–S324

Tan R, Niu X, Gan S, Feng Q (2009) Preparation and characterization of an injectable composite. J Mater Sci Mater Med 20:1245–1253

Tasoglu S, Demirci U (2013) Bioprinting for stem cell research. Trends Biotechnol 31:10–19

Torisawa Y-S, Mosadegh B, Cavnar SP, Ho M, Takayama S (2010) Transwells with microstamped membranes produce micropatterned two-dimensional and three-dimensional co-cultures. Tissue Eng Part C Methods 17:61–67

Torricelli P, Fini M, Rocca M, Giavaresi G, Giardino R (1999) Xenogenic demineralized bone matrix: osteoinduction and influence of associated skeletal defects in heterotopic bone formation in rats. Int Orthop 23:178–181

Turunen S, Haaparanta AM, Aanismaa R, Kellomaki M (2013) Chemical and topographical patterning of hydrogels for neural cell guidance in vitro. J Tissue Eng Regen Med 7:253–270

Vanapalli SA, Duits MH, Mugele F (2009) Microfluidics as a functional tool for cell mechanics. Biomicrofluidics 3:12006

Volponi AA, Pang Y, Sharpe PT (2010) Stem cell-based biological tooth repair and regeneration. Trends Cell Biol 20:715–722

Wang X, Song W, Kawazoe N, Chen G (2013) The osteogenic differentiation of mesenchymal stem cells by controlled cell-cell interaction on micropatterned surfaces. J Biomed Mater Res A 101:3388–3395

Weiner S, Wagner HD (1998) The material bone: structure-mechanical function relations. Ann Rev Mater Sci 28:271–298

Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft lithography in biology and biochemistry. Annu Rev Biomed Eng 3:335–373

Xu F et al (2010) A droplet-based building block approach for bladder smooth muscle cell (SMC) proliferation. Biofabrication 2:014105

Xu T et al (2013) Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. Biofabrication 5:015001

Yasuda K et al (2005) Biomechanical properties of high-toughness double network hydrogels. Biomaterials 26:4468–4475

Yen AH, Sharpe PT (2008) Stem cells and tooth tissue engineering. Cell Tissue Res 331:359–372

Yu T, Ober CK (2003) Methods for the topographical patterning and patterned surface modification of hydrogels based on hydroxyethyl methacrylate. Biomacromolecules 4:1126–1131

Yu J, Shi J, Jin Y (2008) Current approaches and challenges in making a bio-tooth. Tissue Eng Part B Rev 14:307–319

Zorlutuna P et al (2012) Microfabricated biomaterials for engineering 3D tissues. Adv Mater 24:1782–1804

http://www.springer.com/978-3-319-22344-5

Engineering Mineralized and Load Bearing Tissues
Bertassoni, L.E.; Coelho, P.G. (Eds.)
2015, X, 276 p. 60 illus., 54 illus. in color., Hardcover
ISBN: 978-3-319-22344-5