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REVIEW ARTICLE

Fabrication of Polymeric Biomaterials: A Strategy for Tissue Engineering and Medical devices

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Polymeric biomaterials have significant impact in today's health care technology. Polymer hydrogels were the first experimentally designed biomaterials for human use. In this article the design, synthesis and properties of hydrogels, derived from synthetic and natural polymers and their use as biomaterials in tissue engineering are reviewed. The stimuli-responsive hydrogels with controlled degradability and examples of suitable methods for designing such biomaterials, using multidisciplinary approaches from traditional polymer chemistry, materials engineering to molecular biology, have been discussed. Examples of the fabrication of polymer-based biomaterials, utilized for various cells type manipulations for tissue re-generation are also elaborated. Since a highly porous three-dimensional scaffold is crucially important in cellular process, for tissue engineering, recent advances in effective methods of scaffolds fabrication are described. Additionally, the incorporation of factor molecules for the enhancement of tissue formation and their controlled release are also elucidated in this article. Finally, the future challenges in the efficient fabrication of effective polymeric biomaterials in tissue regeneration and medical devices applications.

1. Introduction

The use of polymers as biomaterials have been the subject of intense investigation over the past fifty years.[1,2] Different chemical structures and functional groups in such polymers govern their morphology and properties, and allow precisely control the creation of desired molecular architectures for a wide range of application in the biomedical field. For example, biocompatible polymers have been used successfully as artificial organs and drug delivery systems.[3,4] However, it is to be noted that the degree of success in such applications depend on the self-organization and biocompatibility of the formulated molecular architecture.

The biomaterials which are derived from polymers generally fall into two categories: naturally occurring and human-made synthetic materials. Collagens, alginate and chitosan based materials are the best examples of biomaterials derived from natural resources. The polymers derived from synthetic origins are divided into two classes: non-biodegradable and biodegradable synthetic polymers. Recently, the biodegradable polymers become highly important in the field of biomaterials and tissue engineering, due to the avoidable additional surgery to remove the implants or scaffolds. Thus, much attention needs to be undertaken on the synthesis of biodegradable polymers.

In medical applications there is an on-going research and development (R&D) effort for the improvement of methodologies and devices for more efficient and effective processing of biomaterials. The outcome of such R&D has recently been applied to successfully treat many diseases.[5-7] Amongst the wide range of biomaterials which have been synthesised in recent time for potential use in medicine, majority of these do not have suitable properties to interact effectively with biological tissues or cells. However, it is deemed possible to improve their intrinsic proprieties using required and appropriate process engineering for optimum result. Crosslinking of biopolymers is one of the examples of process engineering which has provided a means to improve the quality of biomaterials for wider medical applications. For example, crosslinked form of soft polymers, classified as hydrogels,[8] is a class of new generation of exciting biomaterials that has demonstrated the ability to form scaffolds for a variety of use such as, tissue engineering, delivery of active molecules, and biosensors and actuators. Hydrogels are 3D structured polymeric materials, "swell gels", which are formed *via* crosslinking reactions of polymers (**Fig. 1**).

The hydrogels can be synthesised with required properties depending on the chemical structure, composition and confirmation of starting materials, density of linking of polymer chains, hydrophobicity and hydrophilicity for a particular biomedical application.

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The 3D structural-integrity and properties of hydrogels are mainly dependent on their method of preparation such as physical or chemical crosslinking reaction.[3,4] Hydrogels from chemical crosslinking form permanent junctions-type networks. The examples of this type of hydrogels include polymerisation of acryloyl group, ionising radiation-induced crosllinking (Photo-polymerisation, **Fig. 1 a**), small molecule crosslinking with polymer chain (glutaraldehyde, **Fig. 1 d**) and polymer-polymer crosslinking by condensation reaction. The physical crosslinking of hydrogels which allow forming transient junctions-type networks, such as polymer chain entanglements or physical interactions (e.g. ionic interactions, as demonstrated in **Fig. 1b**), hydrogen bonds, or hydrophobic interactions. Indeed, there are varieties of different polymer structures which can form physical and chemical hydrogels networks. These polymers structures include linear homopolymers, linear copolymers, and block, random or graft copolymers; polyion-multivalent ion, polyion–polyion or H-bonded complexes; hydrophilic networks stabilized by hydrophobic domains; interpenetrating polymer networks (IPNs) or physical blends; specific molecular recognition; and self-assembling of polymers or polypeptides.

Hydrogels can be synthesised both from natural and synthetic polymers. The examples of hydrogel from natural polymers are: collagen, gelatin, hyalauronic acid, chrondroitin sulphate, chitin and chitosan, alginate, starch, cellulose, and their derivatives. Hydrogels from natural polymers have many advantages over the synthetically derived ones such as low toxicity, good biocompatibility because of their chemical structures are very akin to the structure of glycosaminoglycan (GAG) molecules present in the native extracellular matrix (ECM). Hydrogels from synthetic polymers are prepared by chemical polymerisation methods. Various types of monomers, for examples, acrylates, methacrylates, acrylamides, esters, carboxylic acid and polyfunctional

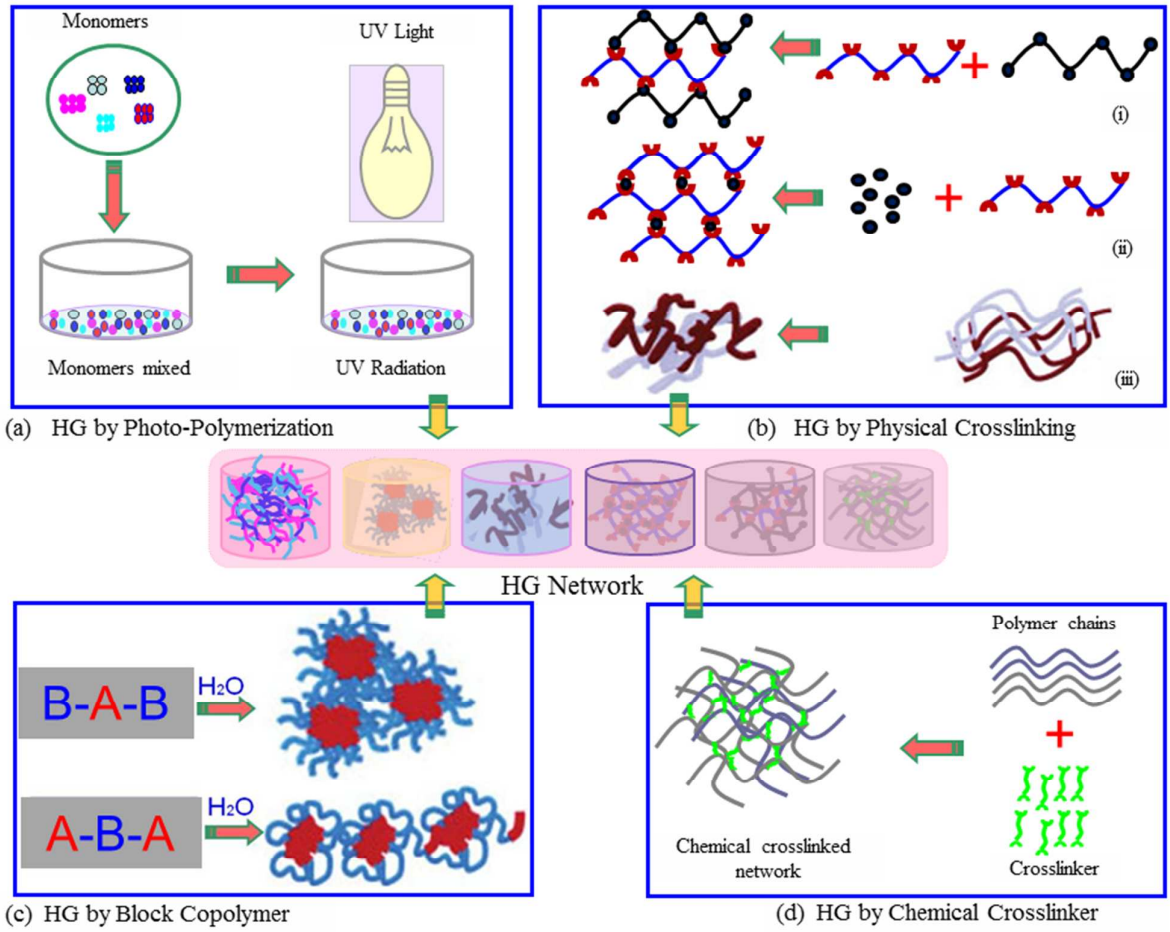

Fig. 1 Schematic representations of the preparation process of polymer hydrogel (HG) using different methods. (a) HG is synthesised by photo-polymerisation. In this the polymer is mixed with appropriate monomers, other formulation components and then irradiated the monomer blend (in vitro or in vivo) with a beam of light of suitable wavelength. (b) HG is produced by physically cross-linking with polymers differently charged (b-i) or with counter ions (b-ii) (e.g., hyaluronic acid, alginate, chitosan), and a polymeric composition may partially crystallize under certain circumstances, and crystallites act as crosslinking point gelling the formulation (b-iii). (c) Block copolymers (BAB, ABA) composed of hydrophobic (A) and hydrophilic (B) units are able to form flower (c, bottom) or core (c, top) micelle when dispersed in water. By increasing polymer concentration or temperature, these micelles are also able to self-assemble in ordered structures that form HG. (d) HG is produced by covalent links between polymeric chains can be created by the use of reactive crosslinker(s) with or without initiators ('chemical' gels).

monomers, can be utilised for the preparation of synthetic hydrogels.[9] The details description of the preparation of hydrogels is beyond the scope of this review. This topic has been covered in depth by several researchers.[9-11]

In this review, we describe the recent developments of polymeric biomaterials and 3D structure generation by utilizing a variety of advanced techniques and methods with emphasis on various types of tissue engineering. Several strategies for the 3D scaffolds fabrication, which include lithography and printing techniques, patterning by self-organisation of polymers, self-assembling peptides, and cellular compatibility of polymer-based biomaterials and hydrogels are presented. The advantages and drawbacks in the 3D scaffold fabrication methods are also discussed. Additionally, we describe the applications of polymeric biomaterials and scaffolds in tissue engineering, particularly to the cartilage, bone and neural tissue regeneration. Furthermore the approaches for incorporation of bioactive factor molecules in biomaterials *via* physical encapsulation and chemical crosslinking, their functions and specific applications in tissue regeneration have been discussed.

2. Tissue Engineering (TE)

The objectives for TE approach is to replace, repair or regenerate damaged tissues, or to create artificial tissues for transplantation, when normal physiologic reaction fails to take place and surgical procedure becomes essential. A number of strategies of TE have been schematically presented in **Fig. 2**. Currently two different standards are used, e.g. autografts and allografts. Each of them, however, has severe limitations, including donor-site morbidity in the case of using autografts and the associated potential risk of disease transmission in the case of using allografts. In recent time, considerable research effort has been made worldwide to overcome the inherent limitations of current standards and to improve the biomedical technology by employing 3D biomaterials scaffold-based TE strategies. In scaffold-based TE approach, it is essential that the interactions of 3D-scaffold materials and cells takes place by means of biocompatibility, cell adhesion, proliferation, growth, differentiation and matrix deposition. Scaffold must be design with an appropriate surface chemistry and morphology to promote cellular functions and with sufficient structural and physical properties such as mechanical strength, porosity and pore sizes. Such scaffolds can be fabricated from the origin of biodegradable and non-biodegradable polymers. In the case of biodegradable 3D scaffold, it must be design in such a way so that it maintains structural integrity, and functions and degrades in a controlled manner, until the new tissues are formed and the function continues.

Biomaterials scaffolds has been synthesised from different types of organic and inorganic polymers and materials including polymers from natural and synthetic origin, ceramics, and their composites. Scaffolds materials must be designed to mimic the 3D structure of native tissue and have the ability to act as delivery agents for growth factors, drugs / antibiotics, and chemotherapeutic agents, depending on the nature of the tissue to be repaired. Biomaterials scaffolds can be pre-fabricated either solid structure or injectable forms that harden in situ (hydrogels) which essentially will depend on the nature of specific tissue engineering application.

3. 3D Scaffolds Fabrication for TE

There are several strategies in TE currently under investigation; examples are schematically described in **Figure 2**. Most of these utilize cells which are seeded onto 3D scaffold. Scaffolds are generally designed to be fabricated with a wide range of properties which include: appropriate surface chemistry, porosity with pore dimension from macro to submicron and interconnectivity networks, which allow cell-cell communication and migration, cell proliferation and differentiation, and finally to maintain the biocompatibility and structural integrity throughout the tissue regeneration process.

Method of fabrication of biocompatible 3D scaffolds with appropriate architectures is divided into two classes: (i) conventional and (ii) rapid prototyping. The former class of fabrication often do not provide sufficient physical and mechanical properties, consequently such type of scaffold undergo deformation because of cells motility. Whereas the rapid prototyping methods do not have such disadvantages and can provide all essential characteristics for specific TE application. 3D nano/micro patterns scaffolds fabricated by rapid prototyping showed significant influence on cellular morphology, cell proliferation and differentiation and also on the functioning of various cell types.[12-14] The scaffold fabrication by conventional methods include phase separation,[15] porogen leaching,[16] gas foaming,[17] fibre meshing[18] and supercritical fluid processing.[19] The second category is more advanced and examples of this prototyping techniques include the selective laser sintering,[20] 3D printing[21] and lithography.[22] More recently, self-organized honeycomb porous structures using block-copolymers[23] have been developed. The following section is highlighted on the recent development on scaffold fabrication by lithography and 3D printing, and also elaborated on self-organization methods, as well as self-assembly of peptides, specifically for the enhancement of cellular functioning in tissue engineering applications.

3.1. 3D Scaffold Fabrication by Lithography and Printing Techniques

Polymer patterning of 3D surfaces in biomedical research to study cellular behaviour and TE [24-26] has generated a great deal of interest within the academic and industrial researchers' world-wide. Because of this, a great deal of advancement has taken place in this technology in recent time, in particular, polymeric biomaterials and crosslinked hydrogels have found wide applications in micro-devices using various approaches. In following section the recent development in hydrogel patterning using photolithography, dip-pen lithography, nanoimprinting, contact printing, solid-free form, robotic deposition and their application in TE have been described.

3.1.1. Photolithography. Photolithography is one of the most well-known fabrication methods in order to generate 3D structure and

pattern using various molecular weights of polymeric materials.[27-40] Photolithographic patterns can be generated in polymer films and in monolayers, for example, in polymer brushes.[41] Site-specific exposure is achieved by illuminating the film through a mask or by using optical interference (holographic) techniques.[42] The interference methods generate periodic patterns such as Bravais lattices.[42] The 3D patterned structures are created by a 'two-steps' method. In the first step, a particular area of a monomer-, oligomer- or polymer-coated surface exposed to ultraviolet (UV) irradiation. This allowed the formation of photopolymerisation, photocrosslinking and /or other chemical reactions such as functionalization and decomposition reactions, or induces phase separation in the exposed areas. In the next step of the process, the remaining polymer surface area which was not exposed to UV radiation remains unreacted and when removed by dissolving in an appropriate solvent it creates a 3D pattern surface, as shown in **Figure 3**.

Photolithography is a high-throughput technique, and is suitable for large-area of 3D surface pattern generation with good alignment (**Fig.3 a-c**) and topography. This technique can provide a broad range of features, varies from micrometres to sub-microns (e.g. 100 nanometres). However, for high-resolution 3D pattern surface generation special type of nonconventional masks, photoactive chemicals (e.g. monomers-, or oligomers or polymers),[43] short wavelengths of radiation, advanced optical techniques and special set-ups for lithographic are needed.[44]

3D pattern surfaces created by this technique are used as

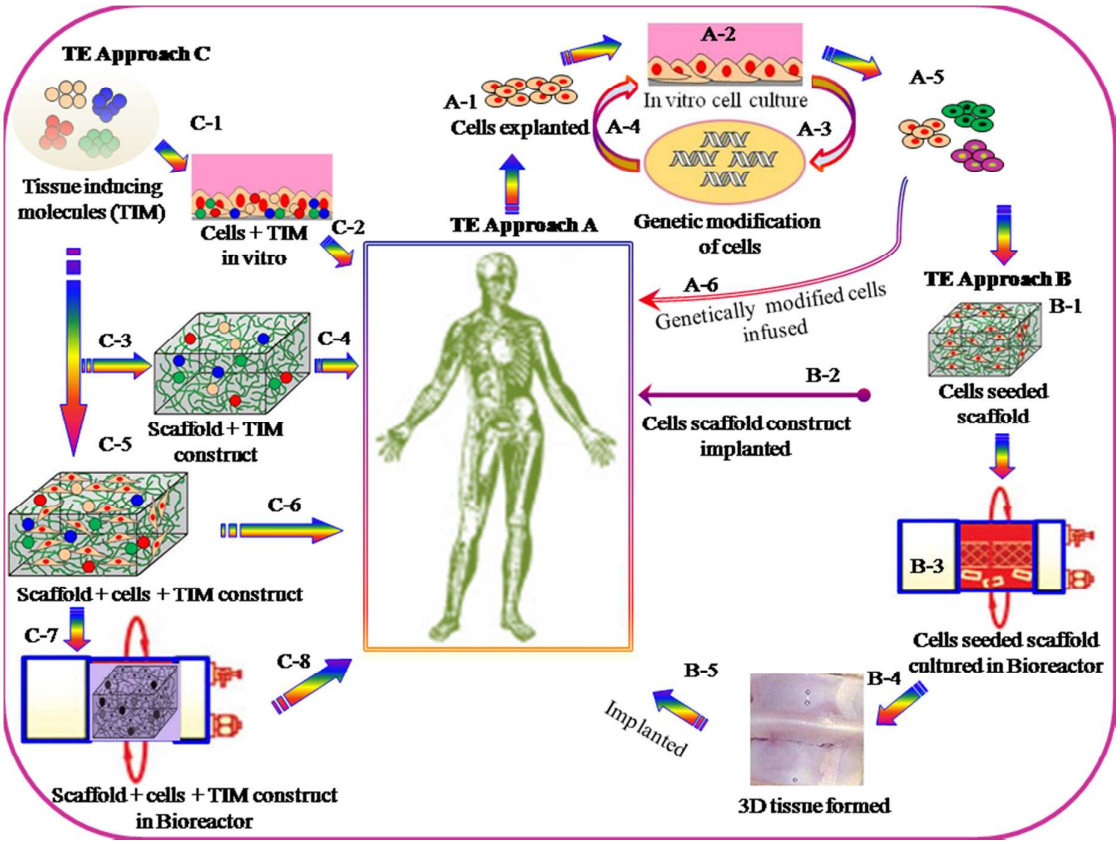

Fig. 2 Schematic representation is showing different tissue engineering (TE) strategies. **TE Approach A:** cells explanted from an individual (A-1), which can be cultivated in vitro (A-2) to differentiate, eventually modify them genetically (A-3, A-4), and expand them (A-5) prior to be reinfused, preferentially, in the same individual (A-6). **TE Approach B:** explanted cells could be engineered before re-exposing them to all the signals (e.g., mechanical, molecular) of the human body. Cells encapsulated or seeded onto the HG /scaffold (B-1) and implanted in the body (B-2) to act as an artificial organ, or cells seeded / encapsulated scaffold assembled in a bioreactor (B-3) to form 3D tissue (B-4) serve as an external artificial organ (i.e. artificial liver), then implanted (B-5). **TE Approach C:** using tissue-inducing substances that can be added in all types of in vitro cultivations (C-1) prior to reinfused to exposed cells in the body (C-2). TIM can be added to the scaffold prior to implantation (C-3, C-4). The use of TIM in vitro and on cells that are growing onto a scaffold (C-5, C-6) that will be implanted after a certain time, or that whole construct can be cultured in bioreactor to generate artificial organ prior to implantation (C-7, C-8).

templates, and subsequently functionalised with other functional materials. Traditionally patterned polymer surfaces are used in the semiconductor industry. In recent years polymer patterned surfaces have found many applications such as LEDs,[45] liquid-crystal displays,[41] photonic crystals,[46] sensors and actuators,[47] and biomedical applications including microarrays of cells, proteins and peptides.[35-39] Here, we focus the use of this technology for cellular application as discussed below.

3D surface patterns that are created by photolithographic process have the ability to manipulate cellular behaviour, and interactions of cells between themselves and with polymer matrix.[32-36] The patterns processed by photolithography provide confine geometry as well as lateral features for cellular adhesion. Due to the multiple features of the patterns this reduces the detrimental effects of cell arrays when cultured for longer time, in contrast to those of other patterning techniques. This method has been employed to create 3D pattern surfaces using chitosan.[37] Karp and co-workers[37] have demonstrated that the generation of 3D patterned surfaces of various shapes (e.g., lanes, squares, triangles and circles) by coating a thin layer of a photocrosslinkable chitosan on a glass slide. Subsequently cardiac fibroblasts were cultured on these patterned surfaces which formed stable patterns for up to 18 days in culture period. Researchers have also demonstrated that when cardiomyocytes were cultured in lanes patterned with 68–99 μm wide, showed expression of cardiac Troponin I and responsive to electrical field stimulation. Osteoblasts (SaOS-2) were also cultured in squares, triangles, or circles (0.063–0.5 mm^2), and the cells were localized in the patterned regions. SaOS-2 proliferated to confluence in 5 days, expressed alkaline phosphatase and produced a mineralized matrix.

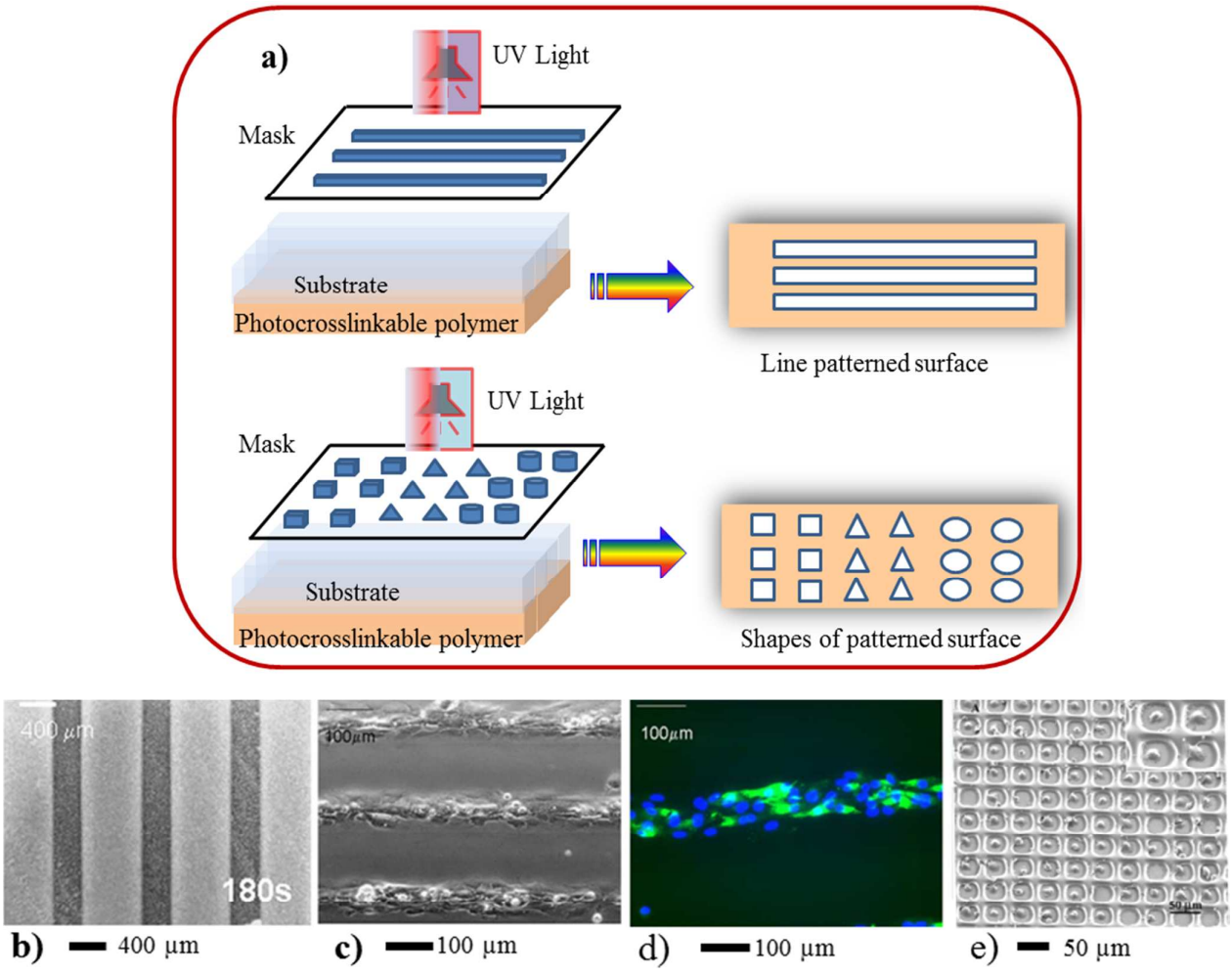

Fig. 3 (a) Scheme represents 3D surface patterning of using photo-induced crosslinakble polymer. In the above illustration (a) mask containing a variety of patterns was placed on top of polymer coated coverslip. UV light was focused onto the mask for a certain period followed by repeated washes in PBS to remove the non-polymerized gel and expose the underlying glass substrate within the patterned regions. (b) A micrograph of a line pattern, (c, d) neonatal rat cardiomyocytes were seeded on chitosan patterned glass surfaces at 8 days of culture. The cells adhered to glass and formed confluent cell lanes that exhibited spontaneous contractions. (d) Patterned cardiomyocytes express cardiac troponin I (green) and exhibit a developed contractile apparatus. (e) Patterning of 3T3 fibroblasts and primary rat hepatocytes in 30×30 μm PEG wells, 10×10 array of single fibroblasts with 91% cell occupancy (×150). The inset shows a higher-magnification image of confined fibroblasts (×1200). (Reproduced with permission.[35] Copyright 2003, ACS.)

Photolithography has also been utilised using a variety of other polymeric biomaterials such as polyethlene glycol (PEG),[39] poly(N-isopropyl acrylamide) (PNIPAAm),[36] and PEG-peptide Arg–Gly–Asp (RGD) hybrid hydrogels[35] for cells patterning and their functional studies.

A high-density Murine 3T3 fibroblasts array was generated (**Fig. 3**), and cells were encapsulated in 3D confined hydrogel micro-wells.[35] Encapsulation of hepatocytes within the PEG-diacrylate hydrogel via photo-induced patterning yielded about 21,000 cell clusters per 100 mm^2 gel as a living cells array with a precise control of cells positioning, in which the duration of cell viability was up to few weeks.[39] Albrecht and co-workers have investigated[40] the multicellular organization in photo-induced patterned 3D hydrogels containing cells viable up to two weeks, which regulates the bovine articular chondrocyte.[40]

However, in photolithographic systems there are some challenges remain to be solved, such as: (i) economic viability of the processing method, (ii) lack of resolution, (iii) lack of original properties following the generation of patterns, and (iv) unsuitability of UV-sensitive biological materials for pattering.

3.1.2. Nanoimprinting Lithography (NIL). NIL is a method for generating economically viable, 3D nano-structured and high-resolution surfaces.[48-55] In this method soft materials, such as polymer, oligomer or monomer formulation (denoted as "resist" in **Fig. 4 a**) are transferred to substrate by pressing mould, subsequently either treated with appropriate temperature, or exposing them to UV radiation, to obtain solid 3D pattern structure, as represented in **Figure 4 a**. The detail description of this method is well documented in several published literatures,[21, 52] therefore only a brief synopsis is presented below.

In this method designing of thermoplastic material to act as a suitable resist is critical factor to obtain high-resolution and defect free 3D pattern surfaces. For example, some polymers such as poly(methyl methacrylate) (PMMA) and polystyrene (PS) are susceptible to lead fracture on the 3D pattern surface.[53] Therefore, multifunctional copolymers, either block or graft- copolymers, are the preferred class of materials for defect free pattern.[55] It has been demonstrated that using polydimethylsiloxane (PDMS)-*block*-PS copolymer as resist a 250 nm line width grating pattern has been constructed **(Fig. 4)**[54] with excellent mould releasing properties and without defect. Another feature of this technology is the formulations using UV-induced polymerisation; particularly those developed using acrylic and methacrylic monomers *via* free radical polymerization due to their high-reactivity. However, the environmental oxygen can lead to detrimental effect to the polymerisation reaction at the surface layer of resist. To resolve this problem, either inert atmosphere during processing or a UV-sensitive cationic crosslinking of cycloaliphatic epoxides has been developed.[55]

Other advanced chemical methods had been adopted for cell-based patterning,[56] tissue engineering[57, 58] and the cellular response to the surface morphology and structures.[59] For these, surface topography has been found to play an important role, as most attached cell types are reactive even in few nm scale differences of topographic structure.[60] Development of groove pattern structures with varying width in the range between 100 nm and 400 nm with a constant depth of 97 nm depths has been reported and this template was utilised for nerve cells guiding.[57] Researchers have demonstrated that cells do not follow the continuity of grooves and ridges, and the pattern surface influenced the shape of the cells by rearranging the cytoskeleton[57] as well as induced gene regulation.[59] Similarly, osteoblasts cells cultured on groove surfaces with a depth of 150 nm, and found similar alignment behaviour of cells.[58] The depth of the groove is a highly important parameter as this determines the wettability of the cells to be aligned.[58]

The challenges such as controlling mould geometry, selection and formulation of thermoplastic resist material, precisely control the process parameters and suitable photosensitive materials selection, are still remained for reproducible of 3D pattern generation, which eventually will dictate future exploitation of this technique in biomedical arena.

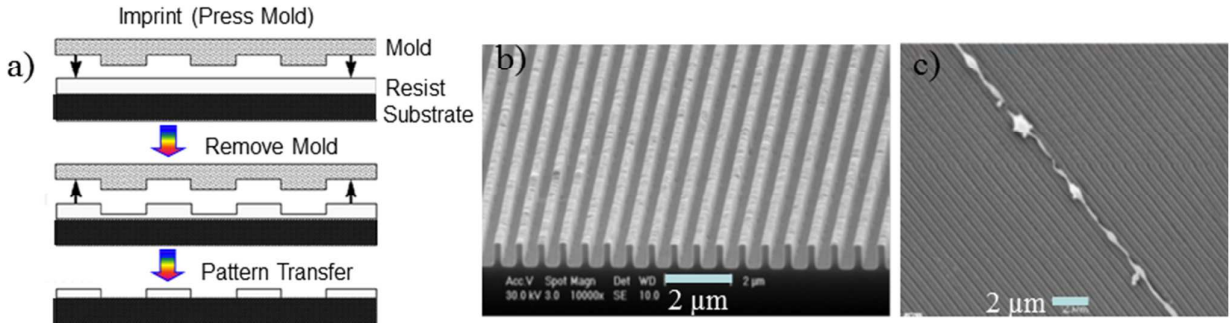

Fig. 4 (a) Scheme of Nanoimprinting. (b) NIL results using a 250 nm line width PDMS-b-PS grating. (c) 400 nm width and 800 nm pitch. SEM images show the axons grow on the ridge edges, and not in the grooves. Reprinted with permission.[54, 58] Copyright 2007 and 2004, Wiley-VCH.

3.1.3. Contact Printing

3.1.3.1. Microcontact printing (μCP) with UV-induced 3D Patterning. Microcontact printing is a remarkable surface patterning technique with spatial resolution down to nano-meter range, developed about a decade ago.[61-66] This technique has drawn enormous attention from communities belonging to materials and chemical science, tissue engineering and biological sciences. In the past few years a significant improvement of the process, particularly, in the design technologies commensurate with biomedical applications.[61] Using this method, a high-quality 3D patterns has been achieved by selecting appropriate conditions with no contamination, without deformation of stamps and the lateral diffusion of the ink, the more details on the μCP patterning have been extensively reviewed in open literature.[22, 61] In μCP a poly(dimethylsiloxane) (PDMS) stamp which has relief features to transfer an inked material to substrate, as demonstrated in **Fig 5. a**. Due to the elastomeric property of PDMS, the stamp deforms macroscopically allowing increasing features over large areas (a few cm^2). PDMS has low surface energy due to the flexibility of the siloxane chain and the low intermolecular forces between the methyl groups,[63] which facilitate peeling of the stamp from the substrate after printing. Researchers have reported patterns with features less than 50 nm using μCP with PDMS stamps.[67] In high-resolution patterning, the deterioration of the surface features could be minimised by using functional polymers that interact with the surface. The examples of such polymers are: poly(acrylic acid), poly(ethyleneimine) and small heavy weight macromolecules (e.g.

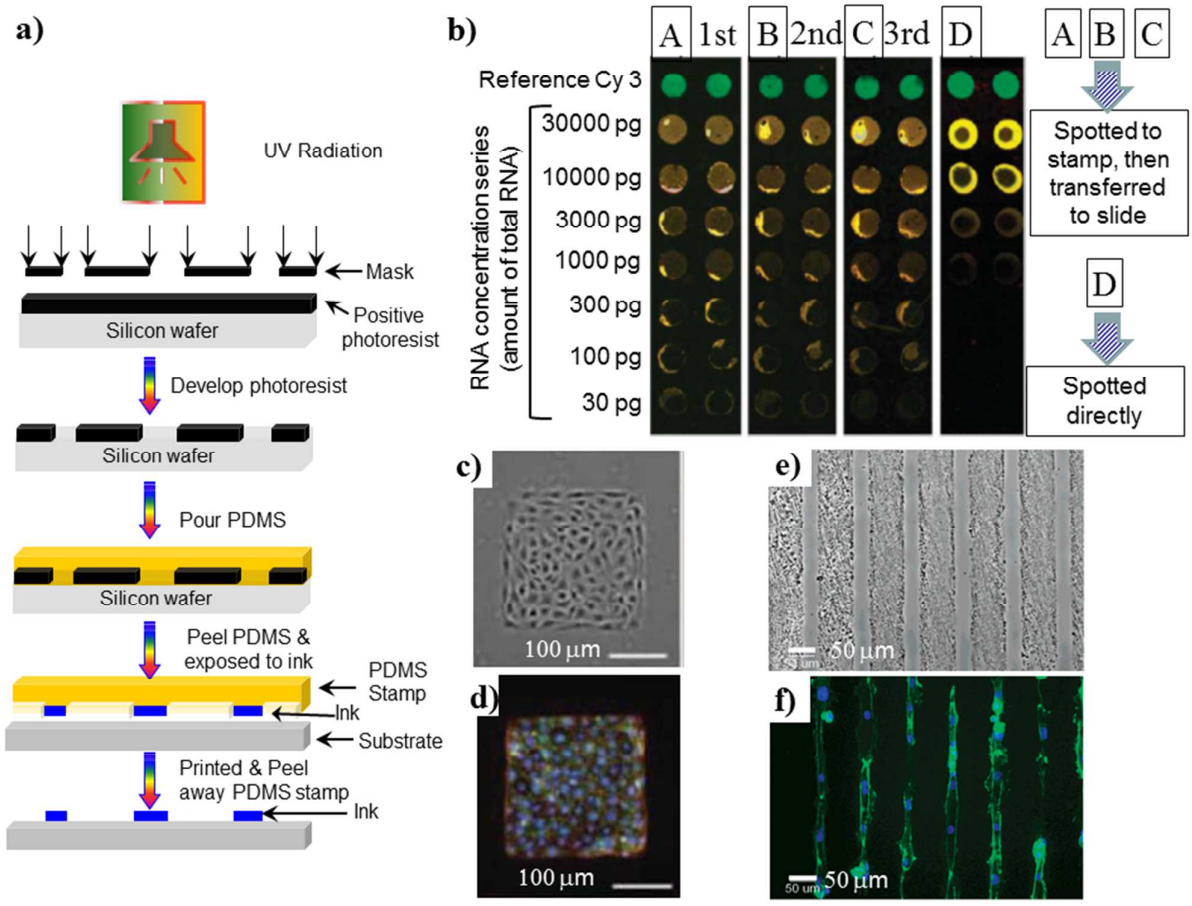

Fig. 5 (a) Schematic representation of microcontact printing. (b) DNA arrays made by μCP a spotted PDMS stamp three in times in succession or by spotting directly. The arrays show hybridizations using different concentrations of RNA starting material. (c, d) A monolayer of bovine pulmonary artery endothelial cells cultured on 250 mm squares of fibronectin. (e) Optical micrograph of OEGMA/MA line pattern on chitosan film, (c) alignment of cytoskeleton and nuclei in NIH3T3 fibroblasts cultured on 30 μm wide lines of PLGA substrates after 24 h. Actin microfilaments (green) were visualized by Alexa 488-labeled phalloidin. Cell nuclei were visualized by DAPI (blue). Reprinted with permission from (b)[68] (Copyright 2004 ACS), (c and d)[69] (Copyright 2005 National Academy of Sciences), (e)[62] (Copyright 2003 ACS) and (f) [73] (Copyright 2005 Elsivier Science).

dendrimers). This technique has been employed enormously in various applications such as plastic electronics, optics, surface sciences and biological fields. For more details on μCP technique the readers are referred to published reviews. [38, 61]

In biological fields, μCP technique has been utilised for patterning DNA,[68] the immobilization of proteins and peptides on substrates for cellular adhesion,[62, 69,70] or protein resistant polymers,[64–66,71] as demonstrated in **Figure 5**. Researchers have demonstrated cell patterning on silicon based substrates,[72] PS,[66] and on bio-resorvable polymers,[62,59] which could potentially be applied in biomedical fields. Several researchers have demonstrated[73-76] that this technique has the ability to manipulate polymeric biomaterials between microns to nanometre scale to obtain various types of patterns shape such as rectangular and lines (**Fig. 5 e**), which has significant positive influence in cellular functioning, regenerative medicine and drug delivery system.

For example, Lin and co-workers created line patterns of proteins and cells using μCP on biodegradable polymers such as poly(lactic acid) (PLA) and poly(lactide-co-glycolide) (PLGA) substrates (**Fig. 5 f**),[73] which are routinely used as scaffolds in tissue engineering. Site-specific immobilisation of proteins and NIH3T3 fibroblasts was achieved by printing a protein resistance polymer such as poly(oligoethylene glycol methacrylate) in a particular area, thus creating line pattern for cellular attachment. Cells remain confined within the line patterns on the PLGA and PLA films for up to 14 days, and aligned their actin cytoskeleton along the line patterns. This suggests that this method could have significant influence in cell-based tissue engineering applications for controlling the spatial morphology and distribution of cells on synthetic biomaterials.

Although this method has few drawbacks such as, multilayer and multicomponent pattern process, which makes it less economically viable. Routinely generated micrometre sizes features using μCP is expected to have an important role in the polymer and biomaterials 3D pattern generation when combining with other techniques,[77-79] e.g. photolithography, dip-pen lithography or with self-assembly polymeric systems.

3.1.3.2. Contact Printing without UV. Microstructure generation by contact printing is a very recent approach used for the deposition of organic solvents onto a solid polymer film surface.[80] The schematic diagram of contact printing by solid pins are shown in Fig. 6 a.[80] In this process two layers of polymer coated glass slide in which bottom layer is chitosan (CS) (thickness ~1 μm) and the top layer is polystyrene surface with thickness either 1.2 μm or 2.4 μm measured by scanning electron microscope were used. The mechanism of microwell fabrication by this technique is totally different than the previously described other techniques (photo- and soft-lithography, μCP, etc.), as in this case the polymer is locally transferred from the centre to the edge region that allowed to form rim very akin to the explanation of micro-fluidic flow proposed for the formation of a "ring-shaped coffee stain" when drop onto a solid surface.[81, 82] This technique has several advantages over other techniques, such as (i) ease of processing, (ii) no bulk flow of solvents required unlike lithography, (iii) the dimensions of microwells can easily be controlled by tuning physical and chemical parameters, and (iv) high density (several hundred e.g. 600 per cm^2) of microwell features can be generated in a single experiment. The well-defined and desired dimension of microwell fabrication will certainly depend on the selection of polymer and its solubility, solvents, sizes of the solid pin, amount of solvent deposited onto polymer surface, printing temperature and humidity. However, the fabrication of microwells in nano-scale range is required to be investigated.

The microwells that have been generated allowed various cell types manipulation, encapsulation and growth,[80] for example, cervical carcinoma (HeLa) and human leukemia (K562) cells, and DNA transfection to the cells have been demonstrated in **Fig. 6**.

This technique is also extensively utilised to fabricate polymer microarrays by dispensing pre-form polymer solution onto a solid surface, which has been described in details in our recent published literatures.[83-85] Such polymer microarrays are immobilised with desire type of cell culture, allowing to indentify cell-compatible polymeric biomaterials for subsequent scaffold fabrication and implantation.[86, 87]

3.1.4. Solid Free-Form 3D Patterning of Polymeric Materials by Ink-Jet Printing. Solid free-form method is an ink-jet printing technique. This has been utilised to generate 3D patterns of polymers onto a substrate either by 'drop-on-demand' or 'continuous' mode, a solution based writing process onto substrates.[88-90] The drop-on-demand systems are subdivided into three categories such as (i) electromechanical (a piezo and electrostatic actuated system), (ii) electrothermal (a thermal actuated system) and (iii) electrostatic vacuum. The continuous mode are divided into two categories such as (i) electric field, e.g., electrical field controlled ink-jet system and (ii) Hertz continuous, a mutual charged droplet repulsion type ink-jet system. In the case former types, signals are used to control the ejection of an individual droplet. While in later systems, ink emerges continuously from a nozzle under pressure, and the jet breaks up into a line of continuous droplets, and the electric signals play a role to control direction of the jet.[91] Both types of ink-jet printing systems can provide features ranging in size from 10 μm to few hundred μm depending on the droplet size, chemical, physical and processing parameters.[92] To achieve precise and reproducible patterning with resolution less than 10 μm is remained challenging. However, the size of features can be reduced by using acoustic and electrohydrodynamic ink-jetting or printing on pre-patterned surfaces. The details on ink-jet printing systems are well reviewed by several research groups.[91,92]

This technique has been utilised in 3D patterning of photoresists, polyelectrolytes, conjugated polymers, biopolymers, photocurable oligomers and monomers, and polymer colloids.

This is a simple method of producing 3D micro-pattern with flexible size and shape. However, it is crucially important particularly for ink-jet printing polymers to identify the well-defined rheological properties of the polymer solutions utilised for patterning, surface tension and the boiling temperature of solvent.[92] In this method the selection of polymer and solvent can be crucial, due to their solubility interaction and viscoelastic properties of polymer

solutions which influence the break-down of jets into droplets.[92] Structure and molecular architecture of polymers, their chain length and polydispersity index, and concentration of polymer in solution will eventually dictates the viscoelastic properties governing the printing pattern. This method has been investigated by research groups to obtain 3D patterning of polymer-arrays using both non-biodegradable[90,93] and biodegradable (PLGA)[88] polymers, for applications in sensors[93] and cells patterning.[88,94,95]

Sanjana and co-workers[95] have generated neuron-adhesive patterns using biodegradable polymer, namely collagen and poly(D-Lysine) (PDL) mixture *via* selectively ink-jet printing on poly(ethylene glycol) (PEG) surface. In these patterns PEG act as cell-repulsive material, while the collagen/PDL mixture act as cells-adhesive material. The inkjet printing technique is used for the construction of synthetic biodegradable scaffolds *via* printing of crosslinker onto liquid alginate/gelatin solutions, which formed 3D hydrogel scaffold that has potential application in tissue engineering.[96]

To achieve a controllable pattern, the substrate on which solvent and polymer are ink-jet printed and the underlying layer should avoid dissolution and swelling.

3.1.5. Robotic Deposition. This is a more advanced technique compared to those described in previous sections. In this method the desired printing materials, e.g. polymer, composite, dispersed materials is continuously deposited onto a substrate either in melts or in solution[97-102] to form a 3D complex surface. In order to obtain heterogeneous 3D structural biomaterials scaffold, parameters such as viscosity of solution, viscoelastic behaviour of biomaterials and their solidifying process after extrusion, along with computer aided

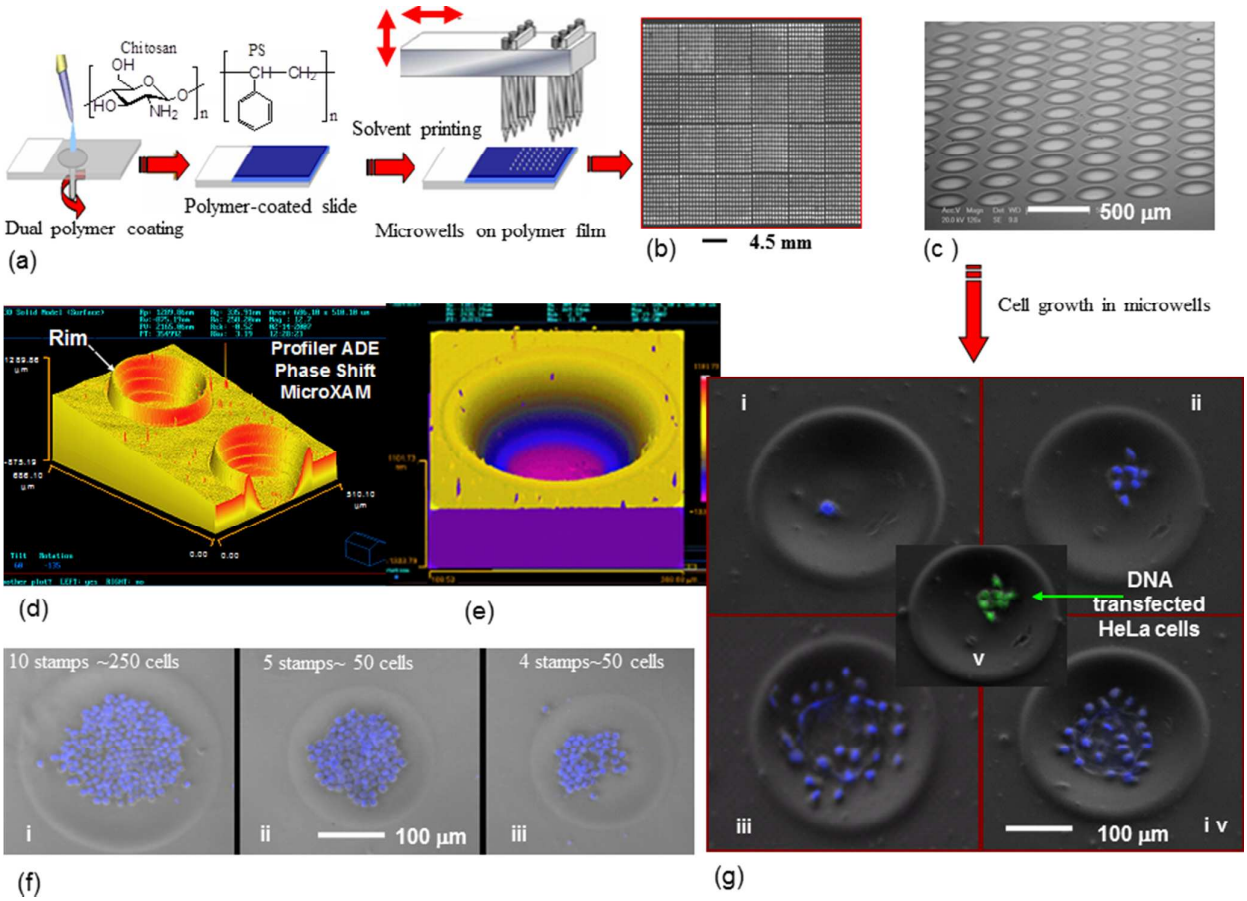

Fig. 6 Microwell fabrication by contact printing and their cellular application: (a) General process of polymer microwell fabrication by contact printing. Images of the fabricated microwell arrays (b and c), b) low-resolution image obtained using a BioAnalyzer 4F/4S white light fluorescent-based scanner showing an array of 600 wells/cm^2 (PS film thickness = 2.4 µm, printing pin diameter 150 µm) and c) SEM image of a microwell array at a 70° angle with 490 wells/cm^2 (PS film thickness = 1.2 µm, printing pin diameter 150 µm). 3D images of microwells fabricated on PS films (d) and (e). (d) Generated using 4 solvent stamps on a 1.2 µm PS film with solid pins of a diameter of 150 µm (K2783). (e) A single microwell fabricated on a PS film (2.4 µm thickness) by stamping acetophenone/ethyl acetate 8 times with a 150 µm diameter solid pin (K2783). (f) Microwells hosting a monolayer of K562 suspension cells. Composite digital image: Phase contrast and DAPI-staining. (g) HeLa cells growth in microwells: culture period 24 h (i), 48 h (ii), 96 h (iii) and (iv), and DNA transfection to HeLa cells (v). Reprinted with permission.[80] Copyright 2007 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

design parameters are needed to be optimised. Using this technique 3D complex architectural scaffold with various pore sizes and porosity can be generated by a computer-control design layer-by-layer printing and solidifying process, as explained in **Fig. 7**.

Many research groups have demonstrated that this technique is very useful for generating complex geometry using various bioactive polymers and copolymers derived from natural and synthetic origins.[101-106] Some examples of such polymers are: poly-L-lactide (PLLA), poly(ε-caprolactone) (PCL),[101] Poly(Lactide-co-Glycolide) (PLGA),[102] poly(ethylene glycol terephthalate-*b*-butylene terephthalate),[103] agarose, gelatin,[104] CS[105] and polyelectrolytes.[106] In the case of polyelectrolytes, the solution blend of cationic and anionic polymers were deposited from a nozzle and rapidly coagulated in an alcohol–water solution to obtain 3D periodic structure. Optical microscopic images of 3D lattices and radial structures with a resolution of 1 μm show high integrity surfaces, as shown in **Fig. 7 b**-d.[107]

This technique has several advantages for 3D heterogeneous structure generation with high efficiency, with features from submicron to micron range in contrast to conventional lithographic methods. Furthermore, this method does not use UV radiation for curing and causes no damage to light-sensitive molecules. Therefore, light sensitive bioactive molecules can be used in the fabrication devices. Several research groups have demonstrated in their published literatures[107-110] that this method can be used to fabricate suitable devices in photonics, microfluidics, bio-mineralization[90-92] and the most promising is the fabrication of scaffolds-matrix for cellular attachment, proliferation and differentiation for tissue regeneration.[110] Seol et al[26] have demonstrated that it is an essential to have appropriate porosity and pore sizes with well-defined shape, mechanical integrity and biocompatibility over a time period for cells to function during tissue regeneration.[26] Articular chondrocytes were cultured on PEG-based block copolymer scaffold for skeletal tissue regeneration. In this system due to the suitable porosity and pore sizes cells were homogenous distributed throughout the scaffolds and supported the formation of the cartilage tissue.[101] A separate investigation[111] showed that when human-bone-marrow-derived osteoprogenitor cells, cultured on scaffolds fabricated using PCL and PCL–hydroxyapatite biodegradable composites, developed osteogenic lineage.[111] As an example, Woodfiled et al[103] have shown that when cellular compatible scaffold, used for tissue regeneration, due to attachment, proliferation of expanded human chondrocytes throughout the scaffold and matrix deposition by the cells led to the filling of pores with high cells viability **(Fig. 7)**.[103]

3.2. 3D-Scaffold Fabrication and Patterning by Self-Organization

As discussed before, there are many techniques and methods have been developed for biomedical applications, particularly to the applications of cell-based tissue engineering and biomedical devices. Each of the above mentioned techniques require multiple steps, highly expensive and limited resources of starting materials needed for scaffolds and devices production. Therefore, there is a need for suitable alternative approaches for 3D structure generation. If we look at our biological nature, certainly one can see a number of examples of fabrication of self-organization of organic and inorganic components at ambient conditions. As an example, one can see butterfly wings has established interference patterns that show the self-cleaning properties similar to leaves and photonic crystals.[112] Another example is the Gecko feet which consists about $5x10^5$ setae and can generate a strong adhesive force which has drawn a significant research interest.[113, 114] Inspired by the 3D pattern exists in biological structures, polymer and biomaterial scientists have developed 3D hierarchy and sophisticated architecture in the order of micron- to nano-structure from functional polymers and biomaterials[112-125] alternative to the existing lithography techniques.

There are many advantages of generating suitable 3D structure by self-organization method. Some examples are as follows: (i) a

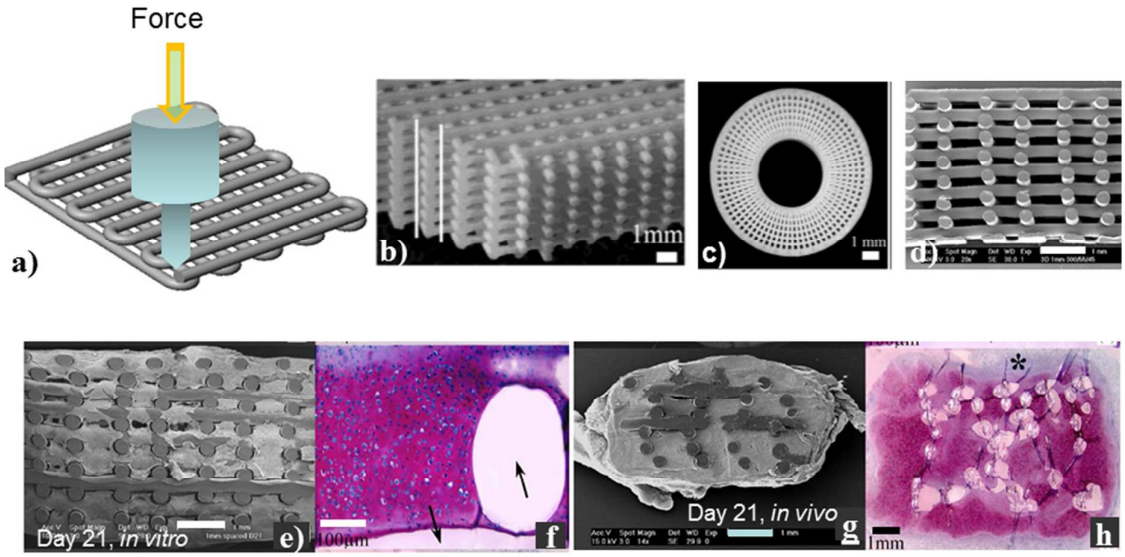

Fig. 7 Robotic deposition (a) two layers scaffold produced by 3D plotting. Optical images illustrating (b) a 3-D periodic structure with a simple tetragonal symmetry reveal the high integrity interfaces formed between layers and (c) a 3D radial structure comprised of alternating layers deposited using radial and concentric fill patterns. (d) SEM section of 3D deposited scaffolds with homogeneous 1 mm fibre spacing showing typical fibre diameter and pore geometries ×20. SEM (e, g) and safranin-O stained (f, h) of 3D-deposited 300/55/45 scaffolds following (e, f) 21 days dynamic culture in vitro; (g, h) 21 days subcutaneous implantation in nude mice; (arrows indicate PEGT/PBT fibre, * indicates fibrous capsule). Scale bar = 1 mm (b, c, d, e, g, h), 100 μm (f). Reprinted with permission.[102, 103] Copyright 2002 and 2004, Elsevier Science.

structure can be generated in physiological condition, (ii) no toxic chemicals or initiators are needed and, (iii) no requirement of high temperature or UV radiation for curing. Therefore, this self-organization technique can be employed in a variety biomedical application. Many research groups have developed reproducible 3D structures of self-organized honeycomb-pattern with highly regular porous networks using a number of different types of polymers under various conditions,[122-132] and their porous network structure have been identified by scanning electron microscopy (SEM), as presented in **Fig. 8 a, b**. This revealed a 3D pattern of highly regular and uniform honeycomb hexagonal pore structure. This structure comprises top and bottom layers, which are laterally interconnected with nano-scale side pores. The tilted SEM image (**Fig. 8b**) clearly shows side-view of two hexagonal lattices connected at the vertices of the hexagons by vertical columns. This double-layered structure reflects the 3D surface morphology of the template, which is a self-organized and hexagonally packed. The mechanisms of these hexagonal structures have been described in several published literatures.[124-127] In brief description, water-immiscible solvent was used to dissolve polymer, followed by the casting of polymer solution onto a substrate surface and then by immediately evaporative cooling of humid air used. This allowed condensation of water droplets to be deposited onto the surface of casted polymer solution. These water droplets acted as a temporary template for pores generation. The condensed water droplets were unstable and it was essential to stabilise water droplets in order to achieve a highly regular honeycomb pattern surface. For achieving water droplet stability, the amphiphilic polymers were used, which act as a surfactant and contribute to the stabilization of the water droplets at the interface of the polymer solution and water, resulting in a highly reproducible and uniform structures. A number of experimental parameters are required to optimise water droplet stabilisation which includes selection of polymer, concentration of polymer in water-immiscible solvent, suitability of solvent and its rate of evaporation, casting volume polymer solution. These parameters ultimately govern the porous network structure, pore sizes and distribution. Researchers[126] have demonstrated that uniform pore size can be achieved by altering the parameters of polymer solution casting. The amount of polymer solution used for casting was found to influence the pore size of the fabricated honeycomb films, because the size of condensed water droplets increased with the evaporation time.

For cell-based biomedical application of 3D porous network structure, it is critically important not only to investigate cellular attachment, viability and growth after culturing on the scaffold matrix, but also other events such as cell spreading, cell migration, and differentiated cell functions. Thus, the physico-chemical and biocompatible properties of 3D scaffold substrate play a significant role in determining the cellular response. It has been demonstrated in several published papers[133-139] that the 3D honeycomb structure of scaffolds have strong influence on cell proliferation, cytoskeleton, focal adhesion, and extracellular protein generation. As an example, hepatocytes formed spheroids, and synthesise albumin and urea when cultured on 3D honeycomb scaffold. Researchers have also found that the pore sizes of the scaffold has significant influence on gene regulation.[132] Culturing of endothelial cells (ECs) on the honeycomb scaffolds with 5 µm pore size allowed to provide high level of proliferation.[100] Similarly neural stem cells (NSCs) was cultured on 3D honeycomb materials with a pore size of 3 µm, and was found to be accelerated proliferation while such 3D structure did not support differentiation of NSCs into neurons.[134] In addition, the pore size of the honeycomb pattern also affects mesenteric-visceral adipocytes function and that a honeycomb film with a pore size of 20 µm had the highest cell functions.

It is interesting to note that studies on the growth of cancer cells on 3D honeycomb surface were also conducted in recent time. It was found that the growth of such cancer cells was much lower as compare to that of a control 2D surface. Hence the surface topography of honeycomb scaffold possibly has an anticancer effect while culturing cancer cells. Thus, the effects of honeycomb structure on cellular phenotypes depend on the cell lineage type, e.g. ECs, NSCs and other normal, cancer and stem cells, and culture conditions. These were achieved in a culturing media which do not contain growth factors. The results of the investigations suggest that the honeycomb structure with different pore sizes could regulate the cell adhesion, morphologies, and functions while no growth factors used. Recently, a vertically open-pored film support for the tubule was given by a metallic tubular mesh was commercialized as a bile duct stent (**Fig. 8 c**). Co-culture of ECs and smooth muscle cells on inner and outer side of the tubular honeycomb film are expected to find applications in novel cardiovascular stents and artificial vessels.

Using a simple method of polymer solution casting on a glass substrate and peeling off adhesive tape a completely different 3D architecture of polymer pincushions arrays can be achieved.[140] Such 3D surfaces, having nano-and micro-structures are suitable for specific cell-based tissue engineering and drug delivery. The mechanical properties and biodegradability of such scaffolds should resemble those of healthy tissues during tissue regeneration. The fabrication of hexagonal arrays of biodegradable polymer pincushions were developed using biodegradable, and biocompatible polymers such as polycaprolactone (PCL), poly(lactic acid) (PLA), poly(D,L-lactide-co-glycolide) (PLGA), and poly(3-hydroxybutyrate) (PHB),[141] which are U.S. Food and Drug Administration (FDA) approved materials. Thus the use of such materials in medical devices can avoid FDA hurdle in clinical application.

The pincushions structures are confirmed by microscopic analysis (**Fig. 8 d, e**), which shows that each pore is surrounded by six pincushions with a diameter of approximately 0.1–0.5 µm. The tilted SEM analysis of pincushion structures (**Fig. 8 f-i**) showed vertically and hairy aligned morphologies. The heights, widths, and distances of separation of the pincushions were dependent on the type of polymer used and the pore size of the original honeycomb film. For example, PCL pincushions (**Fig. 8 f**) showed an elongated hair-like morphology as compared to the pincushions generated from other polymers. Both sharp and hairy pincushion structures could be controlled by peeling off at a certain temperature above or below glass transition temperature. Such structural and morphological differences could be attributed to the polymers viscoelastic and mechanical properties, and their interaction with glass surfaces.

The generation of 3D pincushions was performed under physiological conditions with simplicity, flexibility and cost effectiveness and different from other techniques. It has been demonstrated that the nano-structured surfaces, utilized for long-term maintenance of stem cell phenotype and multipotency,[142] and such structures have positive influence on cells- and materials-based therapeutic applications.[87]

As discussed earlier that honeycomb structure has significantly influence on cellular behaviour as compare to that of flat surface, an example is presented in **Fig. 8 j-q** for endothelial cells growth and differentiation. Immunohistochemical analysis (**Fig. 8 n-q**) revealed that a remarkably high extracellular matrix proteins production when EC cultured on honeycomb film with 5 µm pore surface as compare to that of flat surface.

3.3. 3D Scaffold by Self-Assembly Peptides

Peptides are naturally inspired materials, synthesised from the sequence of the amino acids monomers that carries a carboxyl and an amine functional groups on the chain. The peptides are designed both from natural and synthetic amino acids, they link together to form short peptides then long polypeptide chains in a control manner.[143] Due to the functional groups such as amines (NH) and carbonyls (CO) present in the peptides chain, allow to perform further chemical reactions with functional groups such as thiols and alcohols, and can be combined with a wide range of materials such as lipids, sugars, nucleic acids, metallic nanocrystals and many more.[144] Moreover, the peptides have excellent properties such as biocompatibility, resistance to extreme conditions of high and low temperatures, detergents and denaturants.[144] Thus the peptides

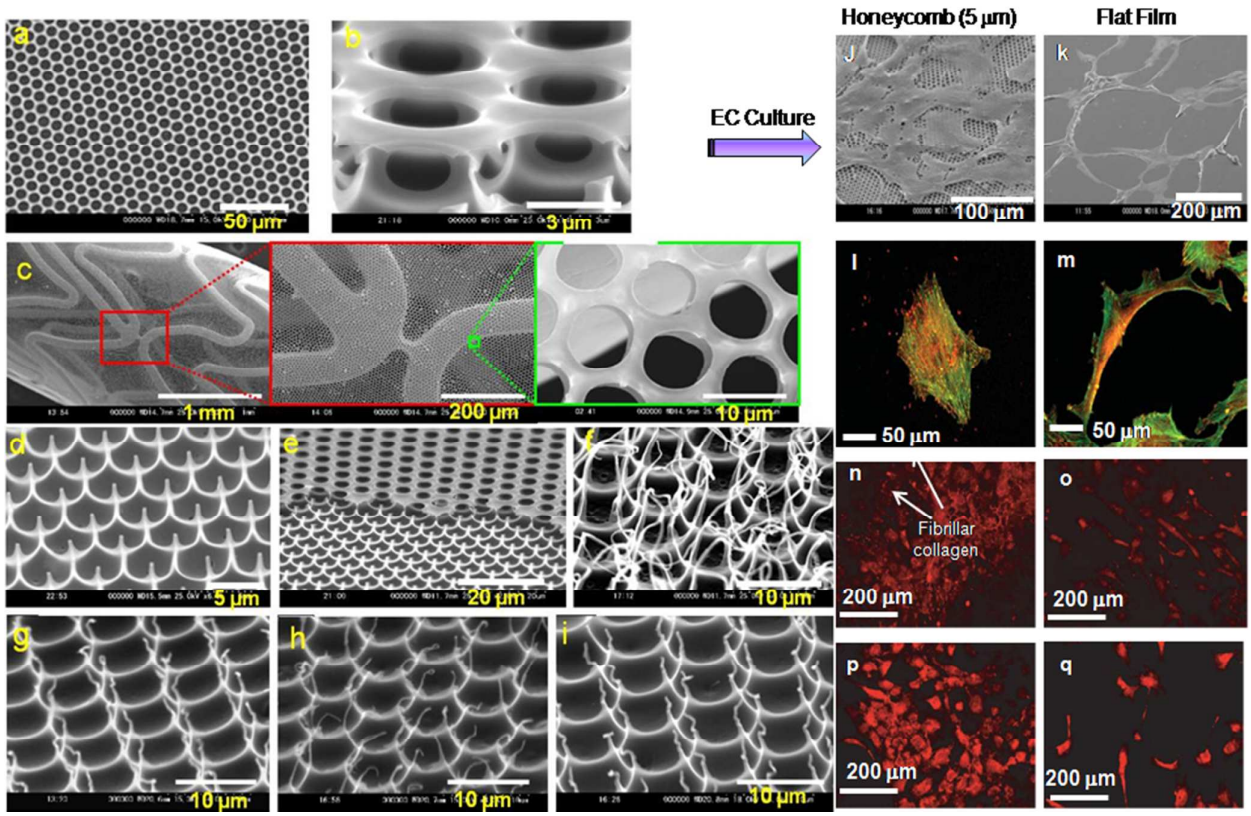

Fig. 8 SEM images of the structure of the self-organized honeycomb pattern surfaces and cellular application: (a, b) regular and uniform hexagonal pores (a) top view and (b) tilted view, (c) bile duct stent covered with a vertically open-pored honeycomb film. SEM images of the surface topography of arrays of polymer pincushions arrays (d - i). (d) Tilt-angle (55°) scanning electron micrographs of the surface topography of arrays of PS pincushion arrays formed on the glass surface. (e) A polymer pincushion surface formed from PTFHMA. Only half of the honeycomb film has been formed into a pincushion pattern. Tilt-angle scanning electron micrographs of the polymer pincushions (f) PCL, (g) PLA, (h) PLGA and (i) PHB.

SEM images of endothelial cells (ECs) cultured on the honeycomb (j) and flat films (k) for 5 days, (l), (m): Cultured on the honeycomb film (pore size, about 5 µm) for 5 days. CLSM images of ECs cultured on honeycomb (l) and flat (m) films. The cytoskeletal protein actin filaments (green) and vinculin (red) are stained using immunofluorescence. Immunofluorescence CLSM images of ECs cultured on the honeycomb films type IV collagen expression (arrow indicates collagen generation) (n), and laminin (p), and on flat film type IV collagen (o) and laminin (q). (a-i) Reprinted with permission.[141] Copyright 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (j-q) Reprinted with permission.[133] Copyright © 2007 American Scientific Publishers.

are capable of wide range of chemical interactions and molecular recognitions, forming various non-covalent interactions in water, including hydrogen bonding, ionic, π—π interactions, hydrophilic and hydrophobic. These interactions lead to the formation of supramolecular self-assemblies that can give rise to a variety of 3D nano-structures such as nano-fibers, nanotubes, and nanoparticles.[145, 146]

In the last two decades, significant advances have been made on the self-assembly peptides (SAPs), and continue to expand rapidly world-wide as a fundamental part of nano-structure generation.[143, 147] Now, these SAPs systems are reaching in a wide range of applications in biology, drug delivery, nanobiotechnology and nanoelectronics.

However, their use in technological applications is facing several challenges, which include (i) the precise positioning of peptide-based nanostructures, (ii) their controlled assembly and positioning, and (iii) their integration into microsystems. Until now, the positioning of the SAPs has been limited on flat surfaces and the fabrication of peptide arrays.

Dinca et al[144] demonstrated that SAPs with unique physical and chemical stability, are capable of functioning as a template for the fabrication of low resistance, and conducting nanowires. In this research, they proposed a methodology for the precise, 3D patterning of amyloid fibrils with combination of laser technology and biotin–avidin mediated assembly on a polymer surface. They also suggested that this method can be applied from molecular electronics to tissue engineering. In this section, we focused the use of SAPs for cells-based tissue regeneration.

In TE, SAPs with low-molecular-weight peptides (oligopeptides) are capable of creating microenvironments suitable for cells culture,[148,149] and tissue regeneration.[150,151] Several researchers[152, 153] have reported that SAP nanofibre scaffolds promoted optic nerve regeneration. These SAPs nanofibre scaffolds are formed spontaneously from individual peptides by interacting with physiological salts and, are entirely biocompatible.[152] Such scaffolds composed of Arg–Ala–Asp–Ala (RADA) oligopeptides utilised in *in vitro* PC12 cells culture which promoted neurite outgrowth and synapse formation by hippocampal neurons.[153]

Kisiday et al [148] have investigated SAP hydrogel constructed with positively charged lysines (K), negatively charged aspartic acids (D) and hydrophobic leucines (L) of twelve units, termed as KLD-12. This hydrogel is utilised for encapsulation of chondrocytes. Chondrocytes seeded within the SAP hydrogel retained their morphology and developed a cartilage-like ECM rich in proteoglycans and type II collagen, in 28 days in vitro culture period. They have also demonstrated that SAPs hydrogel is a potential scaffold for the biosynthesis of extracellular matrix (ECM) and glycosaminoglycan (GAG) accumulation within a 3D cell culture for cartilage tissue repair. The SAPs-based hydrogels can also be used to incorporate bioactive molecules via chemical conjugation to different moieties to allow signaling to cell surface receptors and to enhance cellular adhesion and function.

3.4. Polymeric Biomaterials Mediated Cells Manipulation

Research on various types of cells encapsulation in a variety of polymeric biomaterials, particularly hydrogels derived from matrigel,[154] collagen,[155] alginate[156] and blends of CS and polyethylenimine (PEI)[8] have been investigated. Polymeric scaffolds and biomaterials used in TE to mimic the natural extracellular protein matrix and to provide structural support and cellular functions required for new tissue generation.[157]

Hydrogels are capable of assisting neural regeneration[158] (**Fig. 9 a**), allowing human neural stem cells (hNSCs) to differentiate between neurons and glial cells. The conditions of gel formation are needed to be optimised for Matrigel and PuraMatrix, and mechanical properties are also important for such gel to support hNSCs following transplantation into the injured brain or spinal cord. Several other studies have demonstrated human embryonic stem cells (hESCs) culturing in well-defined 3D settings by using a variety of scaffolds for cellular functioning, cells viability and lineage guidance. The hydrogels synthesised from naturally derived polysaccharides such as hyaluronic acid (HA) supported hESCs growth *in vitro* (**Fig. 9 b**),[155] because it co-regulates gene expression, signalling, proliferation, motility, adhesion, metastasis, and morphogenesis of hESCs *in vivo*. In human, the HA content is greatest in undifferentiated cells and during early embryogenesis and then decreases at the onset of differentiation, where it has a crucial role in regulation of the angiogenic process. It has been demonstrated[155] that when hESC is encapsulated in 3D hydrogels, prepared from HA, hESCs maintained their undifferentiated state (**Fig. 9 b**),[155] and preserved their normal chromosomes state in the cells nuclei. hESCs in hydrogels maintained their full differentiation capacity by embryoid body formation while these cells can be differentiated within the same hydrogel by incorporating soluble factor molecules. Thus HA hydrogels, with their developmentally relevant composition, tuneable porosity, pore sizes and mechanical strength, provide a unique microenvironment for the self-renewal and differentiation of hESCs. The 3D structural biomaterials, developed from synthetic materials, had been tested for self-renewal of hESCs for a limited period. This has shown that much research and development are needed to design robust synthetic materials system associated with relevant bioactive molecules to support long-term of hESCs.

A significant research interests have been drawn both in academia and in biotechnology industries to replace fully or partially biologically derived native materials with synthetics. Materials having biological origins have several drawbacks such as, high cost, batch to batch variation and, sometimes, uncertainty of component identification. While synthetic materials are highly reproducible without variation between batches, and economically viable.

Fischbach et al[159] developed synthetic 3D polymer scaffold to engineer 3D human tumour models using carcinoma cells. Human umbilical vein endothelial cells (HUVEC) were cultured on 2D and 3D surfaces produced from poly(lactide-co-glycolic acid) (PLGA) and matrigels biodegradable polymers, and their analysis was performed by means of proliferation and differentiation. This exhibited angeiogenic potential and cells proliferation of 3D surfaces to be remarkably higher than those from other cultured conditions.[159]

Recently we have demonstrated[8] that water soluble polymer blending of Chitosan and PEI can provide scaffold degradation

behaviour after implantation. Polymer solutions can be mixed with cells before the gelling process can take place allowing cells migration and proliferation throughout the 3D hydrogels scaffold. CS and PEI have been found to support the growth of human fetal skeletal cells within the 3D gel with suitable mechanical properties. The porosity of gels facilitated cell proliferation and prevented dedifferentiation of the skeletal cells into fibroblasts by maintaining these cells in a chondrocyte-like spherical morphology (**Fig. 9 c**).[8]

3.5. Cartilage

Cartilages are tough, flexible tissues which act as shock absorbers. These cover the surface of joints found throughout the body and facilitate bones to slide over one another with reduced friction, and damage. There is no blood supply through cartilage unlike other tissues such as skin or muscle, which makes it difficult to regenerate damaged cartilage tissue. Articular cartilage that lies between joints such as knee joints where the most common and serious damage occurs, resulting in pain, swelling, and some loss of mobility. Therefore, it is essential to develop 3D scaffold matrix for repairing cartilage tissues for clinical applications.

Culturing of mesenchymal stem cells (MSCs) on a variety of TE scaffolds facilitate chondrogenesis and formation of cartilage have been reported in several research papers.[160-164] However, there

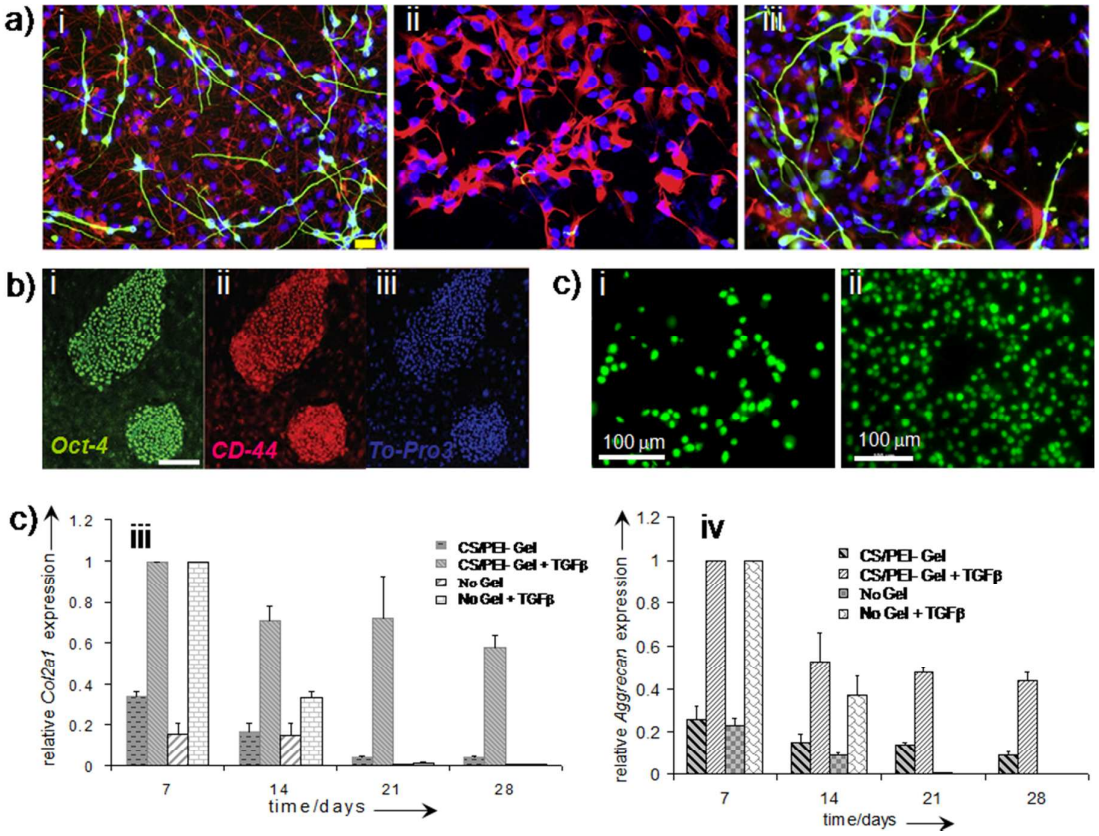

Fig. 9 Polymer hydrogels for cellular function. Differentiation capacity of human neural stem cells beneath Matrigel and PuraMatrix. Immunofluorescent images of human neural stem cells primed for 5 days and then differentiated for 7 days in the absence of a hydrogel (control, a i); in contact with 20% Matrigel (a ii); and in contact with 0.25% PuraMatrix (a iii). Overlay of DAPI (blue), GFAP (red) and Tuj1 (green) and overlay. Scale bar=20 µm (a). Hyaluronic acid (HA) plays a role during hESC culture on MEFs. (b) Staining of hESCs (H1 line) grown on MEFs for HA binding site (green), undifferentiated membrane marker TRA-1–81 (red), and nuclei (blue). Scale bar=100 µm (b).

c) Human fetal skeletal cells, labeled with CellTracker Green, grown in the hydrogel scaffold (chitosan/PEI 40:60): c -i) day 7 and c ii) day 21. Analysis of chondrogenic gene expression (*Col2a1* and *Aggrecan*) by fetal skeletal cells cultured within Chitosan/PEI hydrogels and in monolayers over a course of 28 days with and without TGF-β3. Relative gene expression levels were normalised to the expression of *β-Actin*, which served as a house-keeping gene. The group with the highest expression was assigned a value of 1 and expression levels in the remaining groups were determined relative to that group. Fold relative expression levels were expressed as mean ± SD for plotting as bar graphs, n = 4 for monolayer cultures and n = 3 for hydrogel cultures. (a) Reprinted with permission.[158] Copyright © 2007 Elsevier B.V. (b) Reprinted with permission.[155] Copyright © 2007 by The National Academy of Sciences of the USA. (c) Reprinted with permission,[8] Copyright © 2009 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

appears to have some limitations in achieving identical properties of native cartilages. Additionally, generation of functional cartilage by MSCs has been found to be troublesome, as it depends on the viable cell source for extracellular cartilage matrix production, leading to high quality cartilage regeneration.[164] It is noted that the uniform distribution of such matrix, generated by MSCs is essential for the optimum mechanical strength of the tissue. Therefore, appropriate design of 3D structured biomaterials to support uniform distribution of formed tissue is essential for effective cartilage formation by MSCs.

Studies performed both *in vitro* and *in vivo*[165] have shown that culturing of MSCs on a functionalised HA-based hydrogel by crosslinking method maintains chondrocyte viability and chondrogenic differentiation. It was, however, reported that ECM distribution was not homogeneous due to the unmet degradation rate of hydrogels as a function of ECM production. In an ideal scenario, the tissue engineering of 3D scaffold degradation should match with ECM production by the cells and its accumulation. The degradation rate of scaffolds affects the diffusion of nutrients and waste, cell–cell communication, cell-material interactions, and the distribution and retention of ECM. Therefore, to control rate of degradation of 3D scaffold it is important to select an appropriate crosslinking procedure out of the following: UV-induced crosslinking, chemical crosslinking, and to select appropriate density of crosslinking, or of functional group (if copolymerised) onto the backbone of HA. The MSCs, cultured within HA functionalized hydrogels, showed a rounded cell morphology.[166] It is also reported that the tuning of physical and mechanical properties of scaffolds can control neocartilage formation. For tissue regeneration, the hydrogels scaffolds must control two important properties, i.e. mechanical stability and degradation rate. These can be achieved *via* crosslinking of acrylate and aldehyde groups, which will lead to the repair of cartilage.[167] A modified CS biodegradable hydrogels have been developed,[168, 169] and the biocompatibility was assessed by culturing chondrocytes on the hydrogel scaffold in which cells exhibited clustered growth and produced extracellular matrix on CS gel in *in vitro* condition. This CS gel-chondrocytes promoted cartilage regeneration of defect in rabbits.[168] However, development of hydrogels with high mechanical strength for cell encapsulation and 3D culture is a challenging task for cartilage tissue engineers. Therefore, double network and / or interpenetrating network structures of polymer hydrogels[170-172] are now considered to be potential candidates for cartilage TE.

Polymer substances from natural origin, such as, collagen, alginate, silk fibroin, agarose, etc. were also used to design and fabricate scaffolds in a wide variety of forms including, meshes, sponges, foams, hydrogels, glues, composite layers, biotextiles, nanofibers and microspheres.[171-176] Various synthetic polymeric materials have been used to fabricate scaffolds for cartilage repair. These included PLA, PGA, PLGA copolymers, PEG or PPO polymers. These were found to form gels, ceramic composites and hydrogels containing PEG polymer-based derivatives at different temperatures.[177] A list of polymers scaffolds fabricated by using a variety of techniques and used in pre-clinical animal and clinical human trials in cartilage tissue engineering [178-186] is presented in **Table 1**.

3.6. Bone

The research and development in bone tissue engineering,[187-197] using a combination of cells, factor molecules, and supportive 3D matrices, have gained momentum in recent years. Biomimetic and biodegradable polysaccharides scaffolds derived from chitosan,[198-201] hyaluronic acid (HA),[202-206] and alginate,[207-210] have been developed for bone tissue engineering application. However such materials, in their pure form, have mechanical weakness, instability and lack of remaining predefined shape and thus have limited applications[211] in TE. Therefore, to improve their properties several research groups have developed copolymers,[212,213] blends and composites[214-218] of CS, HA and alginates for bone TE.

Recently, several research groups have reported[219-227] on a variety of biodegradable synthetic polymers scaffolds for bone TE, such scaffolds include PCL,[219-221] poly(lactic acid),[222,223] and their copolymers.[224-229] It has been concluded that these polymer based scaffolds have some advantages over ceramic and glass based ones, primarily because the properties of the polymer based scaffolds can easily be processed tailored to obtain suitable geometry for implantation. The major drawbacks with polymer scaffolds are low mechanical strength and shape retention failure, insufficient cell adhesion and growth, and hence, require surface modification with functional groups or incorporation of bioactive materials to form multicomponent biocompatible composite bone scaffolds[230-243] to enhance osteogenicity[244] for ultimate bone tissue engineering.

Recently very promising polymer based scaffolds have been developed and pre-clinical trials have been conducted (see table 1).[245-250] This showed that, superior biocompatibility, biodegradability and high mechanical strength, and growth factors can be achieved within the scaffold materials to enhance bone formation.[86,87,249,250] Naturally derived polymers, particularly polysaccharides, have found wide application in biomedical technology as signalling molecules such as peptides, proteins can easily be incorporated in these *via* chemical processing. Additionally, these are found to interact well with inorganic components and provide a very akin environment for cells to grow. Gels, crosslinked with inorganic components, lend itself to be processed using a simple chemical processes and can be introduced into the body through a minimally invasive surgery.[189] In recent years, various designed materials construct have been developed in our group, using a blending approach of multi-component polymers for bone tissue engineering.[86,87,249, 250]

3.7. Neural Tissue Engineering (NTE)

Physical injury to the central nervous system (CNS), which can be caused by a severe accidents and neurodegenerative diseases like Parkinson's and Alzheimer's, disrupt brain architecture. As a result severe functional disorder may ensue due to the loss of neuronal cell bodies, axons, and associated glia support. Regeneration of damaged neural tissue, because of their complex structure and functioning, is a highly challenging task in global healthcare system in the field of tissue engineering applications.

Currently autologous nerve grafting approach has been used clinically to repair nerve defects. It is well known that such clinical

Table 1. A list of polymer scaffolds used in pre-clinical animal and human clinical trials.

Materials Types	Scaffold Fabrication Technique	Application	
		Pre-clinical animal trial	Human clinical trial
Poly(L-lactide), Poly(L-lactide-co-glycolide)[177]	Leaching	Cartilage in mice	---
Poly(L-lactide-co-glycolide)/Collagen[178]	Freeze drying	Cartilage in mice	---
Poly(glycolic acid), PCL, Poly(hydroxyl butyrate)[179]	Solvent casting / leaching	Neocartilage in mice	---
Poly(ethylene glycol)-poly(butyl terephthalate) copolymer[180]	Robotic deposition	Cartilage in mice	---
Hyaluronan-based[181]	---	----	Chondrocyte transplantation for cartilage tissue
Collagen, HA, Alginate, PLA[182]	---	---	Cartilage tissue
Hyaluronan-based[184, 185]	---	---	Chondrocyte implantation; transplantation.
Collagen[244]	Freeze-drying	Tibia defects /bone in rats	---
Collagen/hyaluronate[245]	Cross-linking	Cranial defects /bone in rats	---
PLGA/Poly(vinyl alcohol)[246]	Leaching	Cranial defects/bone in rabbits	---
Poly(propylene glycol-co-fumaric acid)[247]	Gas foaming with effervescent reaction (in vivo)	Cortical defects bone in rats	---
PCL/PLLA[179, 248-250]	Solvent evaporation	Trabecular bone in mice and sheep	---
CS/Poly(vinyl acetate)/PLLA blends[87]	Freeze drying	Trabecular bone in mice	
Gelatin[277]	Leaching	Artificial skin in mice	---
Poly(ethylene glycol), cystamine and PCL[280]	Crosslinking	Connective tissue in rats	---
Silk[281]	Freeze drying	Ligament in pig.	---

approach has two major disadvantages: (i) loss of function in the donor nerve graft sensory distribution and (ii) geometrical mismatch between the damaged nerve and the nerve graft. Thus, there is a need for neural TE strategy to be developed, focusing on 3D scaffolds generation with a favourable neural cells growth that in facilitates regeneration. Several researchers have utilised scaffolds for enhancing regeneration within the CNS, and generated promising results.[251] With the aim of nerve regeneration, several research groups have, independently, developed a variety of polymeric templates.[251-259] For example, Tsai et al[251] have synthesised poly(2-hydroxyethyl methacrylate) (PHEMA)-methyl methacrylate (MMA) hydrogel, which enables to incorporate growth factor molecules. This copolymer system combining with growth factors has allowed spinal cord injury repairing in animal models.

Recent studied suggested that stimuli responsive soft materials, especially electrically stimulated hydrogels, have played significant role in the proliferation and differentiation of nerve cells.[252-254] The neurite extension and outgrowth was substantially enhanced on electrically conducting polymer hydrogels in different culturing media. The effect was found to be more prominent in negatively charged polymeric materials than in positively charged or neutral[252] ones. The neutral polymeric hydrogels (e.g. PEG, PHEMA), functionalized with ionic compounds to form ionic hydrogels, are able to bridge a spinal cord lesion when implanted inside a hemi-section cavity. HEMA-based hydrogels with charged functional groups, either cationic or anionic, have the ability to enhance axonal regeneration inside the implant, and surprisingly, no charge was observed when minimal axons infiltrate hydrogels.[253] Researchers have also found that implanted hydrogels with

positively charged groups increased axonal ingrowth into the central part of the implant. Astrocytes infiltrate only those hydrogel implants comprising negative charge or neutral group, most of which are found only in the peripheral zones. Functional groups on the backbone of HEMA hydrogel with different surface charges and density of charge influence the interaction between cells and materials and cellular functioning[254] and consequently improve the quality of nerve regeneration. Therefore, conductive polymer-based materials-aligned scaffolds[260-272] and the incorporation of carbon-based nanomaterials[270-277] into polymeric scaffolds have been investigated for neural tissue growth. Such acrylate-based hydrogel polymers are classified as non-biodegradable materials, lack of desirable feature to be used in TE as a scaffold.

Biodegradability of polymer scaffolds plays an important role in TE. They act as a temporary scaffold holding the growing tissue in place until the natural ECM has sufficiently developed. The scaffold breakdowns into nontoxic degradable products those are capable of being disposed of by the body and leaving behind the newly formed tissue. There are a number of natural and synthetic biodegradable polymers such as collagen, HA, chitin and chitosan, PLLA and PLGA that are explored as scaffolds for NTE application.[278, 279]

Biocompatible polymeric hydrogels and scaffolds have also been investigated for regeneration of various other tissues, as shown in **Table 1**, such as artificial skin,[280] connective tissue and ligaments.[281, 282]

3.8. Growth factors (GFs) incorporated in hydrogel and angiogenesis

A growth factor is defined as a naturally occurring protein or steroid hormone that binds to specific receptors on the surface of their target cells. GFs can play a role in a variety of physiological processes such as, new blood vessel generation, phenotypic activities of cells, tissue development and healing, wound healing and treatment of myocardial and hind limb ischemia.[283-292] However, the stability of GFs is critical factor in the above processes when administered *in vivo*. Therefore, suitable delivery system to improve stability is needed in order to promote neo-vascularization at a local tissue site. The hydrogel polymer has been found to influence controlled release of such factor molecules.[293,294]

For optimum performance of GFs, it is necessary to combine these with carrier molecules in order to release it in a control manner. Although some success of current clinically available GFs delivery devices have been reported in some TE fields, these are not even near enough to an ideal system, demanding further research on efficient and sustainable delivery devices. The clinical technologist and the researchers within the biotechnology industry are enthusiastically looking for systems for controlled and efficient delivery using lower dose of GFs and for the production of a more sustained release pattern to serve as a more effective 3D scaffold surface with structural support in tissue engineering. An in-depth understanding of tissue-healing processes is, therefore, needed to allow us design new suitable delivery systems for GFs. Additionally, the processes of normal tissue-healing needed to be biologically optimized so that there are sequential overlapping stages for the transition from immature to mature (definite) tissues. Logically, mimicking both the structures and the sequence of the tissue-healing process should be the best option for designing biomaterials for TE. This is mainly because of their ability to initiate the body's natural tissue-healing cascades at the site of injury. A number of GFs that have been studied in biomedical applications to enhance TE and angiogenesis in recent time are included in **Table 2**.

Polymeric hydrogels play a significant role as ECM scaffolds by serving as a matrix for bioactive molecules delivery to the cells as well as regulating cellular activity. The GF can be incorporated within the hydrogel matrix by crosslinking during the preparation of the gel and can control the sustain release, as demonstrated in **Fig. 10**. Polymer hydrogels are highly porous network structure through which cell migration, cell proliferation and cell-cell communication take place. Lowe and co-worker[293] described that cells communicate with the ECM *via* signalling pathways through integrin which eventually can alter gene expression, resulting in cell migration, differentiation, proliferation or apoptosis. Several studies[157, 295] show that vascular endothelial growth factor (VEGF) is one of the most important growth factors for repairing many types of tissues. Other examples of GFs are bone morphogenic proteins such as, BMP-2 and BMP-7, which have shown bone formation in clinical use.[296, 297] However, such factors still remains unsafe[298, 299] and highly expensive.[300]

Protein based hydrogels have been used to deliver bone morphogenetic protein 2 (BMP-2) for skull defect.[301] However the main disadvantages of the protein based hydrogels are the control of their degradation as most of them are derived from animal products, such as metrigels. Therefore, current research has been devoted to the development of synthetic based polymer hydrogels. A class of synthetic based materials, namely injectable polymer hydrogels derived from the oligo(poly(ethylene glycol) fumarate) (OPF), developed by Park and co-worker,[302] for the delivery of GFs to the cells. The hydrolysis of OPF hydrogels can degrade to the ester bonds in the fumarate group.

Recently, the thermo-reversible polymer hydrogels have attracted a considerable attention both in academia and industry, particularly in TE scaffold technology and drug delivery.[303] Cell cultured in thermo-reversible hydrogels demonstrated higher viability and enhanced cellular functions.[297] The research has shown that the thermo-reversible polymers were useful as an injectable hydrogels. However, very limited study in '*in vivo*' tests for TE, involving growth factors incorporation has been conducted so far.[304] Therefore, an injectable, in situ crosslinkable, biodegradable and thermo-reversible, hydrogel is needed for minimally invasive delivery of therapeutic molecules to the localised cells and tissues. The *in situ* crosslinking approach of injectable hydrogels, with or without cells into the infracted myocardium, shows improvement in neovascularization and heart function and enhanced angiogenic response.[292] In this research, Researchers reported that injectable alginate-based hydrogels with and without RGD - modified alginate, increased the arteriole density as compared to that of control one with the RGD modified alginate having the greatest angiogenic

Table 2. A number of growth factors which can be incorporated in polymer hydrogels and scaffolds to promote various tissue regeneration.

Growth Factors	Tissue engineering application	Function
Ang-1[283]	Blood vessel, heart, muscle	Promote maturation and stability of blood vessel.
Ang-2 [283]	Blood vessel	Destabilize, regress and disassociate ECs from surrounding tissues.
BMP-2 [283, 296, 297, 301]	Bone, cartilage	Promote differentiation and migration of osteoblasts.
BMP-7[283, 296, 297]	Bone, cartilage, kidney	Enhance differentiation and migration of osteoblasts, as well as renal development.
BMP-9[307]	Bone	Enhance osteogenic differentiation and bone formation.
EGF[283]	Dermal tissue, epithelia tissue, nerve healing	Maintaining epithelial cell growth, proliferation and differentiation.
FGF-2[283]	Blood vessel, bone, skin, nerve, spine, muscle	Induce angiogenesis; enhance the formation of blood vessels; migration, proliferation and survival of ECs; inhibition of differentiation of embryonic stem cells.
EPO [283]	Nerve, spine, wound healing	Promoting the survival of red blood cells and development of precursors to red blood cells. Protect myocardium from ischemic injury.
G-CSF[308]	Prevents common chemotherapy complications	Peripheral artery disease and critical limb ischemia.
GM-CSF[308]	Epithelial tissues	Circulating leukocytes, act as a paracrine fashion to recruit circulating neutrophils, monocytes and lymphocytes to enhance their functions in host defence.
HGF [283]	Bone, liver, muscle	Promoting proliferation, migration and differentiation of MSCs.
IGF-1[283]	Muscle, bone, cartilage, liver, lung, kidney, nerve, skin	Cell proliferation and inhibition of cell apoptosis.
GDF-5[309]	Central nervous system	Increasing the survival of neurons.
GDF-8 (myostatin)[310]	Muscle	Control the growth and differentiation of muscle cells.
GDF-9 [311]	----------	Regulating ovarian follicular growth.
GDF-10[312]	Skeletal	Skeletal morphogenesis.
GDF-11[313]	Muscle	Regenerative capacity of satellite cells.
NGF [283]	nerve, spine, brain	Survival and proliferation of neural cells.
PDGF-AB (or BB) [283]	Blood vessel, muscle, bone, cartilage, skin	Function for embryonic development, proliferation, migration, growth of ECs.
TGF- α[283]	Brain, skin	Assisting proliferation of basal cells or neural cells.
TGF- β[283]	Bone, cartilage	Enhancing proliferation and differentiation of bone-forming cells, antiproliferative factor for epithelial cells.
TPO[314]	Liver	Hepatic progenitors during fetal liver development.
VEGF[283, 295]	Blood vessel	Migration, proliferation and survival of ECs.

Note: Ang (angiopoietin), BMP (bone morphogenetic protein), EGF (epidermal growth factor), EPO (erythropoietin), FGF (fibroblast growth factor), G-CSF (granulocyte-colony stimulating factor), GM-CSF (granulocyte-macrophage colony-stimulating factor), HGF (hepatocyte growth factor), IGF (insulin-like growth factor), GDF (growth and differentiation factor), NGF (nerve growth factor), PDGF (platelet-derived growth factor), TGF (transforming growth factor), TPO (thrombopoietin) and VEGF (vascular endothelial growth factor).

response. In this case alginate biopolymers act as a synthetic ECM and RGD peptides as a cell-matrix mediator, which increase cell binding affinity and effect on cell behaviours through integrin–ligand interactions. Several pre-clinical studies have demonstrated that angiogenic GFs can stimulate the development of collateral arteries in animal models of peripheral and myocardial ischemia.[303]

An *in vivo* study shows that the CS based hydrogels could be useful for gradually release of the fibroblast growth factor-2 (FGF-2) molecules as they biodegraded *in vivo.*[304] Researchers also described that releasing FGF-2 molecules from the hydrogels caused induction of angiogenesis and collateral circulation occurred in healing impaired diabetic (*db/db)* mice and in the ischemic limbs of rats. However the sustain release and local delivery of GF will certainly depend on the nature and characteristics of the hydrogel and the method of fabrication and process. The GFs can be crosslinked with the gel materials either physical interactions or chemical reactions. Recently it has been demonstrated that photo-induced crosslinkable and biodegradable pluronic/heparin composite hydrogels were synthesised with a specific objectives for local and sustained delivery of basic fibroblast growth factor (bFGF) to induce angiogenesis.[305]

However, there are still several concerns that GFs have adverse effects, especially for using high level of doses. Exposure of myocardium to high local levels of GFs can cause hemangioma-like tumors, vascular malformations, and neointimal development.[306] To minimize such adverse effects, dose reduction of GFs and their control delivery would be an important strategy in this field.

4. Conclusions and Future Prospects

A numerous efforts have been made globally in the last two decades to bring laboratory-based ideas into clinical trial stage followed by clinical procedures in biomedical applications. Currently biomaterials technology, within the overall healthcare system is receiving benefits as a result of multidisciplinary field of research, albeit often with disappointing output. For achieving the ultimate goal, however, many challenges need to be addressed and overcome. For TE applications, the production of more complex scaffolds materials with biomimetic properties and mechanical stability is necessary. Designing and fabrication of biomimetic synthetic scaffold, aimed at producing biofunctional synthetic matrices to enhance cellular function and leading to high quality tissues development, are also issues to be addressed. Combining multiple physical and chemical approaches with incorporating suitable functionalities to the molecular chain of polymeric materials is expected to provide complex scaffold with architectural-hierarchy, which will enable to mimic the cellular environment, exchange information with cells and enhance cell-cell communication.

To date, the examples of technologically advanced biomaterials have been the multi-component polymer hydrogels derived from a various functional monomers, polymers or oligomers, synthesized either by physical or chemical crosslinking. Such hydrogel systems are expected to find potential use in variety of areas including the regeneration of tissues, and the delivery of bioactive molecules (e.g. growth factors, drugs). However, a number of hurdles, such as biocompatibility, mechanical strength, rate of degradation, etc. also need to be addressed and overcome for effective TE applications. For such applications, the materials with controllable mechanical properties, degradation profiles and 3D structure which could easily be modified to suit a particular tissue purpose need to be developed. Now we can generate 3D synthetic scaffolds with appropriate structural properties that actively support cells to form tissue in defect site. For example, the polymer scaffolds have been found to generate new bone formation when implanted into a defect site in rat femora without the use of expensive growth factors.[86,87,249,250] Naturally derived polymeric biomaterials employed in a wide range of applications, some device materials are commercially available and such polymers most likely to remain best candidate for further research and evaluation in biotechnology. In the future, the research is expected to continue on the processing of 3D structure and product development using naturally derived polymers, perhaps with combining this with synthetic polymers, for appropriate tissue type.

To generate 3D structure of scaffolds, various production techniques and methods using different types of polymeric materials need to be employed. Each technique has a particular processing method and multiple steps and has advantages and disadvantages. Therefore, the choice of technique for 3D fabrication will depend on the nature and type of materials, their structure and properties, interaction with substrate and finally, the intended applications. The robotic technique is considered to be the best choice in an application where a complex 3D architecture scaffold is needed as the generation of such structure is not possible by other techniques. However, some of which are still in an early stage of development and require significant improvement in the bio-chemical technology as well as an in-depth understanding of the basic processes involved. The latter will provide tools to generate structures with best performance. The challenges are the improvement of 3D patterning efficiency with high resolution and low-cost pattering, with good level of performance. To achieve cost-effective 3D patterning and to achieve patterns in multiple length scales, a combination of different techniques will be necessary. Recently, self-organization techniques have shown very promising approach for the cost-effective fabrication of 3D honeycomb structure with exiting *in vitro* and *in vivo* results, but more research is need in this area. Self-assembly peptides nanostructures are very promising biomimetic materials and could be used in various field of TE, but still in their early stage of development. Incorporation of bioactive molecules into the 3D polymer hydrogels/scaffolds and their release in a control manner towards targeted tissue will provide a powerful methodology to study and manipulate developmental and regenerative processes. This will depend on the biological demand of the target tissue.

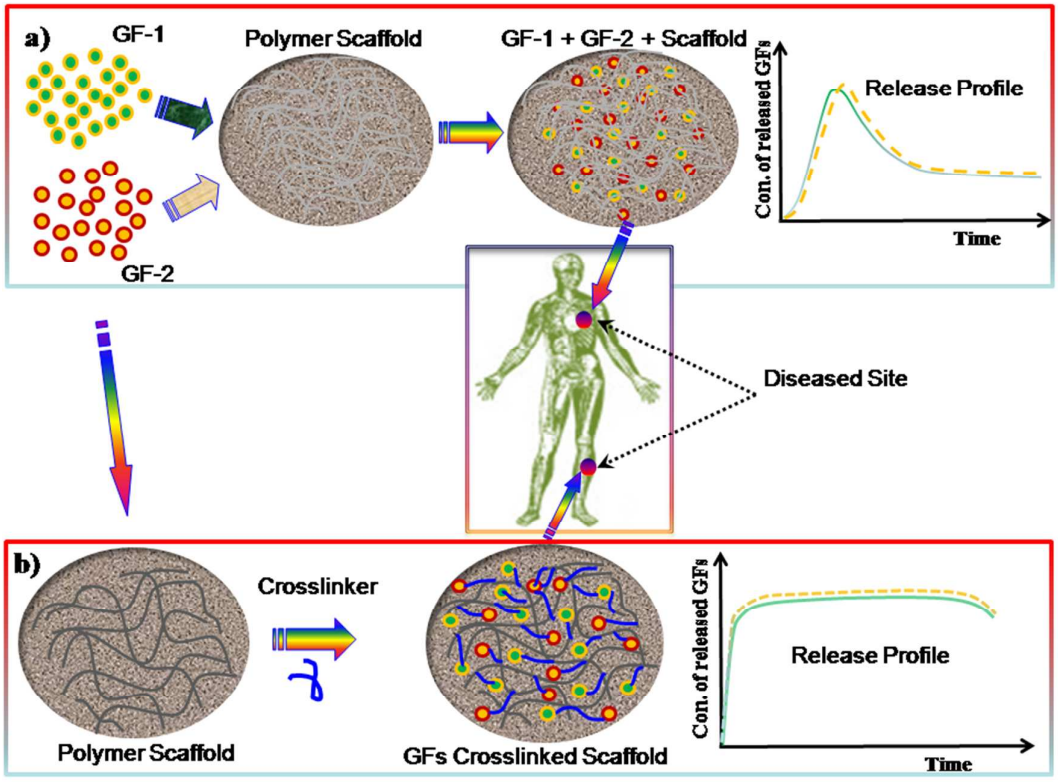

Fig. 10. Schematic illustration of methods for immobilization of bioactive factor molecules (growth factors) into hydrogels scaffolds. (a) Non-covalent immobilization two different types of growth factors (GFs) are loaded into hydrogels directly *via* in entrapment prior to implantation and their expected release profile. (b) Covalent immobilization of two types of GFs are modified and thereafter covalently crosslinked to the hydrogels via crosslinker prior to implantation to diseased site and their sustain release profile.

Harnessing the potential of this technology for clinical use strongly depends upon more researchers and multidisciplinary approaches that combine engineering, biomaterials, medicine and the technical expertise of medical specialists. Working in close collaboration between polymer chemists, materials scientists, tissue engineers and reconstructive surgeons may eventually help to achieve clinical excellence and products for a range of degenerative diseases, for ultimate improvement in the quality of life.

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Notes and references

1 O. Wichterle and D. Lim, *Nature,* 1960, **185**, 117.
2 I. C. Kwon, Y. H. Bae and S. W. Kim, *Nature,* 1991, **354**, 291.
3 A. S. Hoffman, *Adv. Drug. Deliv. Rev.,* 2002, **43**, 3.
4 D. Shi, *Introduction to Biomaterials.* World Scientific: Tsinghua University Press, 2006.
5 W. G. Liu, M. Griffith and F. Li, *J. Mater. Sci.: Mater. Med.,* 2008, **19**, 3365.
6 F. Yang, Y. Wang, Z. Zhang, B. Hsu, E. W. Jabs and J. H. Elisseeff, *Bone,* 2008, **43**, 55.
7 E. Eljarrat-Binstock, F. Orucov, J. Frucht-Pery, J. Pe'er and A. J. Domb, *J. Ocular. Pharm. Ther.,* 2008, **24**, 344.
8 F. Khan, R. S. Tare, R. O. C. Oreffo and M. Bradley, *Angew. Chem. Int. Ed.,* 2009, **48**, 978.
9 C. R. Nuttelman, M. A. Rice, A. E. Rydholm, C. N. Salinas, D. N. Shah and K. S. Anseth, *Progress Polym. Sci.,* 2008, **33,** 167.
10 W.E. Hennink and C. F. van Nostrum, *Adv. Drug Del. Rev.* 2002, **54,** 13.
11 K. R. Kamath and K. Park, *Adv. Drug Del. Rev.* 1993, **11,** 59.
12 S. J. Hollister, *Nat. Mater.,* 2005, **4,** 518.
13 A. S. G. Curtis and C.D.W. Wilkinson, *Biomaterials,* 1997,**18**,1573.
14 M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. W. Wilkinson and R. O. C. Oreffo, *Nat. Mater.,* 2007, **6**, 997.
15 A. Asefnejad, M. T. Khorasani, A. Behnamghader, B. Farsadzadeh and S. Bonakdar, *Int. J. Nanomed.,* 2011, **6**, 2375.
16 R. M. Allaf and I. V. Rivero, *J. Mater. Sci.: Mater. Med.,* 2011, **22**, 1843.
17 C. Z. Wang, M. L. Ho, W. C. Chen, C. C. Chiu, Y. L. Hung, C. K. Wang and S. C. Wu, *Mater. Sci. Eng. C: Mater. Biolog. Appl.,* 2011, **31**, 1141.
18 A. Saraf, L. S. Baggett, R. M. Raphael, F. K. Kasper and A. G. Mikos, *J. Controlled Release,* 2010, **143**, 95.

19 E. Reverchon, S. Cardea and C. Rapuano, *J. Supercrit. Fluids.,* 2008, **45**, 365.
20 B. Duan, M. Wang, W. Y. Zhou, W. L. Cheung, Z. Y. Li and W. W. Lu, *Acta Biomater.,* 2010, **6,** 4495.
21 F. Khan, S. R. Ahmad, in *Handbook of Biomaterials and Stem Cells in Regenerative Medicine* (Editors: M. Ramalingam, S. Ramakrishna, S. Best), CRC Press, Taylor & Francis USA, 2012, Ch 5, p101-121.
22 B. D. Gates, Q. Xu, M. Stewart, D. Ryan, C. G. Willson and G. M. Whitesides, *Chem. Rev.,* 2005, **105**, 1171.
23 G. Widawski, M. Rawiso and B. Francois, *Nature,* 1994, **369**, 387.
24 M. Théry, V. Racine, A. Pépin, M. Piel, Y. Chen, J.–B. Sibarita and M. Bornens, *Nat. Cell. Biol.,* 2005, **7**, 947.
25 M. Théry, V. Racine, M. Piel, A. Pépin, A. Dimitrov, Y. Chen, S. J. B. Sibarita and M. Bornens, *Proc. Natl. Acad. Sci. USA,* 2006, **103**, 19771.
26 Y. J. Seol, T. Y. Kang and D. W. Cho, *Soft Matter.,* 2012, **8,** 1730.
27 M. R. Beaulieu, N. R. Hendricks and J. J. Watkins, *ACS Photonics*, 2014, **1**,799.
28 S. Park, D. Kim, S. Y. Ko, J. O. Park, S. Akella, B. Xu, Y. Zhang and S. Fraden, *Lab on Chip,* 2014, **14***,* 1551.
29 G. Comina, A. Suska and D. Filippini, *Lab Chip* 2014, **14***,* 424.
30 R. Voelkel, U. Vogler, A. Bramati, M. Hennemeyer, R. Zoberbier, A. Voigt, G. Grutzner, N. Unal and U. Hofmann, *Microsystem Technologies,* 2014, **20***,* 1839.
31 M. Muller, J. Becher, M. Schnabelrauch, M. Zenobi-Wong, *J. Visual. Experiments,* 2012, **67,** e50632/1.
32 J. J. Balowski, Y. Wang and N. L. Allbritton, *Adv. Mater.,* 2013, **25***,* 4107.
33 Y. Lu and S. Chen, *Methods Molecular Biology,* 2012, **868**, 289.
34 M. A. K. Liebschner, M. Wettergreen, *Methods Molecular Biology,* 2012, **868**, 71.
35 A. Revzin, R. G. Tompkins and M. Toner, *Langmuir,* 2003, **19**, 9855.
36 M. Yamato, C. Konno, M. Utsumi, A. Kikuchi and T. Okano, *Biomaterials,* 2002, **23**, 561.
37 J. M. Karp, Y. Yeo, W. Geng, C. Cannizarro, K. Yan, D. S. Kohane, G. Vunjak-Novakovic, R. S. Langer and M. Radisic, *Biomaterials,* 2006, **27**, 4755.
38 E. Menard, M. A. Meitl, Y. Sun, J. U. Park, D. J. L. Shir, Y. S. Nam, S. Jeon and J. A. Rogers, *Chem. Rev.,* 2007, **107**, 1117.
39 D. R. Albrecht, V. L. Tsang, R. L. Sah and S. N. Bhatia, *Lab Chip,* 2005, **5**, 111.
40 D. R. Albrecht, G. H. Underhill, T. B. Wassermann, R. L. Sah and S. N. Bhatia, *Nat. Methods,* 2006, **3,** 369.
41 E. Menard, M. A. Meitl, Y. Sun, J. U. Park, D. J. L. Shir, Y.-S. Nam, S. Jeon and J. A. Rogers, *Chem. Rev.,* 2007, **107**, 1117.
42 J. H. Moon, J. Ford and S. Yang, *Polym. Adv. Technol.,* 2006, **17**, 83.
43 T. M. Bloomstein, M. Rothschild, R. R. Kunz, D. E. Hardy, R. B. Goodman and S. T. Palmacci, *J. Vac. Sci. Technol. B*, 1998, **16**, 3154.
44 T. M. Bloomstein, M. F. Marchant, S. Deneault, D. E. Hardy and M. Rothschild, *Opt. Express*, 2006, **14**, 6434.
45 C. D. Müller, A. Falcou, N. Reckefuss, M. Rojahn, V. Wiederhirn, P. Rudati, H. Frohne, O. Nuyken, H. Becker and K. Meerholz, *Nature,* 2003, **421**, 829.
46 M. Campbell, D. N. Sharp, M. T. Harrison, R. G. Denning and A. G. Turberfield, *Nature,* 2000, **404,** 53.
47 J. Hoffmann, M. Plotner, D. Kuckling and W. J. Fischer, *Sens. Actuat. A*, 1999, **77,** 139.
48 Z. Liu, D. G. Bucknall and M. G. Allen, *J. Micromech. Microeng.,* 2011, **21***,* 065036/1.
49 Z. Liu, D. G. Bucknall and M. G. Allen, *Nanotechnology,* 2011, **22***,* 225302/1.
50 D. Xu, K. P. Chen, K. Ohlinger and Y. Lin, *Nanotechnology,* 2011, **22**, 035303/1.
51 Z. Zhendong, B. Benfeng; D. Huigao, Z. Haosu, Z. Mingqian, Y. Oubo, L. Qunqing, T. Qiaofeng, W. Jia and F. Shoushan, *Small,* 2014, **10***,* 1603.
52 T. Chen, I. Amin and R. Jordan, *Chem. Soc. Rev.,* 2012, **41***,* 3280.
53 C. Chao and L. J. Guo, *J. Vac. Sci. Technol. B,* 2002, **20**, 832.
54 L. J. Guo, *Adv. Mater.,* 2007, **19**, 495.
55 X. Cheng, L. J. Guo and P. F. Fu, *Adv. Mater.,* 2005, **17**, 1419.
56 R. S. Kane, S. Takayama, E. Ostuni, D. E. Ingber and G. M. Whitesides, *Biomaterials,* 1999, **20,** 2363.
57 F. Johansson, P. Carlberg, N. Danielsen, L. Montelius and M. Kanje, *Biomaterials,* 2006, **27**, 1251.
58 S. Lenhert, L. Zhang, J. Mueller, H. P. Wiesmann, G. Erker, H. Fuchs and L. Chi, *Adv. Mater.,* 2004, **16,** 619.
59 M. J. Dalby, M. O. Riehle, S. J. Yarwood, C. D. W. Wilkinson and A. S. G. Curtis, *Exp. Cell. Res.,* 2003, **81**, 274.
60 A. S. G. Curtis, *Eur. Cells. Mater.,* 2004, **8**, 27.
61 A. P. Quist, E. Pavlovic and S. Oscarsson, *Anal. Bioanal. Chem.,* 2005, **381**, 591.
62 G. Kumar, Y. C. Wang, C. Co and C. C. Ho, *Langmuir,* 2003, **19***,* 10550.
63 J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo and G. M. Whitesides, *Chem. Rev.,* 2005, **105**, 1103.
64 J. Hyun, H. Ma , Z. Zhang, T. Beebe and A. Chilkoti, *Adv. Mater.,* 2003, **15**, 576.
65 A. Khademhosseini, S. Jon, K. Y. Suh, T. N. T. Tran, G. Eng, J. Yeh, J. Seong and R. Langer, *Adv. Mater.,* 2003, **15***,* 1995.
66 V. A. Liu, W. E. Jastromb and S. N. Bhatia, *J. Biomed. Mater. Res.,* 2002, **60***,* 126.
67 H. W. Li, B. V. O. Muir, G. Fichet and W. T. S. Huck, *Langmuir,* 2003, **19**, 1963.
68 S. A. Lange, V. Benes, D. P. Kern, J. K. H. Horber and A. Bernard, *Anal. Chem.,* 2004, **76**, 1641.
69 C. M. Nelson, R. P. Jean, J. L. Tan, W. F. Liu, N. J. Sniadecki, A. A. Spector and C. S. Chen, *Proc. Natl. Acad. Sci. U. S. A.,* 2005, **102,** 11594.
70 J. M. Corey and E. L. Feldman, *Exp. Neurol.* 2003, 184, Suppl 1, S89
71 M. Mrksich, L. E. Dike, J. Tien, D. E. Ingber and G. M. Whitesides, *Exp. Cell. Res.,* 1997, **235***,* 305.
72 M. Scholl, C. Sprossler, M. Denyer, M. Krause, K. Nakajima, A. Maelicke, W.Knoll and A. Offenhausser, *J. Neurosci. Methods,* 2000, **104***,* 65.
73 C. C. Lin, C. C. Co and C. C. Ho, *Biomaterials,* 2005, **26**, 3655.
74 H. Bae, H. Chu, F. Edalat, J. M. Cha, S. Sant, A. Kashyap, A. F. Ahari, C. H. Kwon, J. W. Nichol and S. Manoucheri, *J. Tissue Eng. Regen. Med.,* 2014, **8***,* 1.
75 H. Hwang, J. Park, C. Shin, Y. K. Do and Y. K. Cho, *Biomedical Microdevices,* 2013, **15,** 627.
76 M. F. Hsieh, C. Sheu and H. Yang, *Adv. Mater. Res.,* 2013, **647**, 170.
77 S. Danmark, M. Gladnikoff, T. Frisk, M. Zelenina, K. Mustafa, A. Russom and A. Finne-Wistrand, *Biomed. Microdevices,* 2012, **14,** 885.
78 A. P. Zhang, X. Qu, P. Soman, K. C. Hribar, J. W. Lee, S. Chen and S. He, *Adv. Mater.,* 2012, **24**, 4266.
79 A. Koroleva, A. A. Gill, I. Ortega, J. W. Haycock, S. Schlie, S. D.Gittard, B. N. Chichkov and F. Claeyssens, *Biofabrication,* 2012, **4***,* 025005/1.
80 F. Khan, R. Zhang, A. Unciti-Broceta, J. J. Diaz-Mochon and M. Bradly, *Adv. Mater.,* 2007, **19**, 3524.
81 E. Bonaccurso, H. J. Butt, B. Hankeln, B. Niesenhaus, K. Graf, *Appl. Phys. Lett.,* 2005, **89**, 124101.
82 R. D. Deegan, O. Bakaljin, T. F. Dupont, G. Huber, S. R. Nagel, T. A. Witten, *Nature*, 1997, **389**, 827.

83 C. N. Medine, F. Khan, S. Pernagallo, R. Zhang, O. Tura, M. Bradley and D. C. Hay, in *Handbook of Biomaterials and Stem Cells in Regenerative Medicine* (Editors: M. Ramalingam, S. Ramakrishna, S. Best), CRC Press, Taylor & Francis USA, 2012, Ch 1, p1-30.
84 R. S. Tare, F. Khan, G. Tourniaire, S. M. Morgan, M. Bradley and R. O. C. Oreffo, *Biomaterials*, 2009, **30**, 1045.
85 F. Khan, S. Valere, S. Fuhrmann, V. Arrighi and M. Bradley, *J. Mater. Chem. B*, 2013, **1**, 2590.
86 F. Khan, R. S. Tare, J. M. Kanczler, R. O. C. Oreffo, M. Bradley, *Biomaterials,* 2010, **31,** 2216.
87 F. Khan, J. O. Smith, J. M. Kanzcler, R. S. Tare, R. O. C. Oreffo and M. Bradley, *Adv. Funct. Mater.,* 2013, **23**, 2850.
88 J. D. Kim, J. S. Choi, B. S. Kim, Y. C. Choi and Y. W. Cho, *Polymer,* 2010, **51**, 2147.
89 B. J. de Gans, P. C. Duineveld and U. S. Schubert, *Adv. Mater.,* 2004, **16**, 203.
90 R. Zhang, A. Liberski, F. Khan, J. J. Diaz-Mochon and M. Bradley, *Chem. Commun.,*2008, 1317.
91 T. Billiet, M. Vandenhaute, J. Schelfhout, S. V. Vlierberghe, P. Dubruel, *Biomaterials,* 2012, **33**, 6020.
92 Y. Christanti and L. M. Walker, *J. Non-Newtonian Fluid Mech.,* 2001, **100**, 9.
93 J. C. Carter, R. M. Alvis, S. B. Brown, K. C. Langry, T. S. Wilson, M. T. McBride, M. L. Myrick, W. R. Cox, M. E. Grove and B. W. Colston, *Biosens. Bioelectron.,* 2006, **21**, 1359.
94 E. A. Roth, T. Xu, M. Das, C. Cregory, J. J. Hickman and T. Boland, *Biomaterials,* 2004, **25,** 3707.
95 N.E. Sanjana and S. B. Fuller, *J. Neurosci. Meth.* 2004, **136,** 151.
96 T. Boland, X. Tao, B. J. Damon, B. Manley, P. Kesari, S. Jalota, S. Bhaduri, *Mat. Sci. Eng. C,* 2007, **27**, 372.
97 S. Z. Guo, M. C. Heuzey and D. Therriault, *Langmuir,* 2014, **30**, 1142.
98 C. Gao, M. N. Rahaman, Q. Gao, A. Teramoto and K. Abe, *J. Biomedical Mater. Res. Part A,* 2013, **101A**, 2027.
99 H. Yun, S. Kim, Y. Hyun, S. Heo and J. Shin, *Chem. Mater.,* 2007, **19**, 6363.
100 M. Xu, G. M. Gratson, E. B. Duoss, R. F. Shepherd and J. A. Lewis, *Soft Matter,* 2006, **2**, 205.
101 G. Vozzi, A. Previti, D. De Rossi and A. Ahluwalia, *Tissue Eng.,* 2002, **8**, 1089.
102 G. Vozzi, C. Flaim, A. Ahluwaliaand S. Bhatia, *Biomaterials,* 2003, **24**, 2533.
103 T. B. F. Woodfield, J. Malda, J. deWijn, F. Peters, J. Riesle and C. A. van Blitterswijk, *Biomaterials,* 2004, **25**, 4149.
104 R. Landers, U. Hubner, R. Schmelzeisen and R. Mulhaupt, *Biomaterials,* 2002, **23**, 4437.
105 L. Geng, W. Feng, D. W. Hutmacher, Y. S. Wong, H. T. Loh and J. Y. H. Fuh, *Rapid Prototyping J.,* 2005, **11**, 90.
106 G. M. Gratson, M. Xu and J. A. Lewis, *Nature,* 2004, **428**, 386.
107 D. Therriault, S. R. White and J. A. Lewis, *Nat. Mater.,* 2003, **2,** 62.
108 M. Xu, G. M. Gratson, E. B. Duoss, R. F. Shepherd and J. A. Lewis, *Soft Matter.,* 2006, **2**, 205.
109 G. M. Gratson, F. Garcia-Santamaria, V. Lousse, M. Xu, S. Fan, J. A. Lewis and P. V. Braun, *Adv. Mater.*, 2006, **18**, 461.
110 D. W. Hutmacher, *Biomaterials,* 2000, **21**, 2529.
111 M. Endres, D. W. Hutmacher, A. J. Salgado, C. Kaps, J. Ringe, R. L. Reis, M. Sittinger, A. Braddwood and J. T. Schantz, *Tissue Eng.,* 2003, **9**, 689.
112 A. Campo and E. Arzt, *Chem. Rev.,* 2008, **108**, 911.
113 P. Vukusic and J. P. Sambles, *Nature,* 2003, **424**, 852.
114 K. Autumn, Y. A. Liang, S. T. Hsieh, W. Sesch, W. P. Chan, T. W. Kenny, R. Fearing and R. J. Full, *Nature,* 2000, **405**, 681.
115 R. M. Duffy, A. W. Feinberg and W. Adam, *Nanomed. Nanobiotech.,* 2014, **6**, 178.
116 M. R. Badrossamay, K. Balachandran, A. K. Capulli, H. M. Golecki, A. Agarwal, J. A. Goss, H. Kim, K. Shin and K. K. Parker, *Biomaterials,* 2014, **35**, 3188.
117 M. Koepf, F. Cherioux, J. A. Wytko and J. Weiss, *Coordination Chem. Rev.,* 2012, **256**, 2872.
118 H. Aubin, J. W. Nichol, C. B. Hutson, H. Bae, A. L. Sieminski, D. M. Cropek, P. Akhyari and A. Khademhosseini, *Biomaterials,* 2010, **31**, 6941.
119 C. Suwanchawalit, A. J. Patil, R. K. Kumar, S. Wongnawa and S. Mann, *J. Mater. Chem.,* 2009, **19**, 8478.
120 Z. Nie and E. Kumacheva, *Nat. Mater.,* 2008, **7**, 74.
121 A. Mahdavi, L. Ferreira, C. Sundback, J. W. Nicho, E. P. Chan, D. J. D. Carter, et al. *Proc. Natl. Acad. Sci. USA,* 2008, **105**, 2307.
122 J. R. McMillan, M. Akiyama, M. Tanaka, S. Yamamoto, M. Goto, R. Abe, D. Sawamura, M. Shimomura and H. Shimizu, *Tissue Eng.,* 2007, **13**, 789.
123 M. Birch, M. Tanaka, G. Kirmizidis, S. Yamamoto and M. Shimomura, *Tissue Eng. A*, 2013, **19**, 2087.
124 H. Choi, M. Tanaka, T. Hiragun, M. Hide and K. Sugimoto, *Nanomedicine,* 2014, **10**, 313.
125 J. O. Eniwumide, M.Tanaka, N. Nagai, Y. Morita, J.De Bruijn , S. Yamamoto, S. Onodera, E. Kondo, K. Yasuda and M. Shimomura, *BioMed Res. Int.,* 2014, in press.
126 M. Tanaka, M. Takebayashi, M. Miyama, J. Nishida and M. Shimomura, *Biomed. Mater. Eng.,* 2004, **14**, 439.
127 Y. Fukuhira, E. Kitazono, T. Hayashi, H. Kaneko, M. Tanaka, M. Shimomura and Y. Sumi, *Biomaterials,* 2006, **27**, 1797.
128 N. Maruyama, T. Koito, T. Sawadaishi, O. Karthaus, K. Ijiro, N. Nishi and M. Shimomura, *Thin Solid Films,* 1998, **327-329**, 854.
129 M. H. Stenzel-Rosenbaum, T. P. Davis, A. G. Fane and V. Chen, *Angew. Chem. Int. Ed.,* 2001, **40**, 1408.
130 H. Yabu, M. Tanaka, K. Ijiro and M. Shimomura, *Langmuir,* 2003, **19**, 694.
131 M. Tanaka, K. Yoshizawa, A. Tsuruma, H. Sunami, S. Yamamoto and M. Shimomura, *Colloids Surfaces A: Physicochem. Eng. Aspects.,* 2008, **313-314**, 515.
132 Y. Fukuhira, M. Ito, H. Kaneko, Y. Sumi, M. Tanaka, S. Yamamoto and M. Shimomura, *J. Biomed. Mater. Res. Part B: Appl. Biomater.,* 2008, **86B,** 353.
133 M. Tanaka, A. Takayama, E. Ito, H. Sunami, S. Yamamoto and M. Shimomura, *J. Nanosci. Nanotech.,* 2007, **7,** 763.
134 M. Tanaka, K. Nishikawa, H. Okubo, H. Kamachi, T. Kawai, M. Matsushita, S. Todo and M. Shimomura, *Colloids Surfaces A: Physicochem. Eng. Aspects,* 2006, **284–285**, 464.
135 M. Shimomura, T. Nishikawa, A. Mochizuki and M. Tanaka, *JP.* 2001/157574.
136 A. Tsuruma, M. Tanaka, S. Yamamoto and M. Shimomura, *Colloids Surfaces A: Physicochem. Eng. Aspects,* 2008, **313-314**, 536.
137 S. Yamamoto, M. Tanaka, H. Sunami, S. Yamashita, Y. Morita and M. Shimomura, *Langmuir,* 2007, **23**, 8114.
138 H. Ishihata, M. Tanaka, N. Iwama, M. Ara, M. Shimonishi, M. Nagamine, N. Murakami, S. Kanaya, E. Nemoto, H. Shimauchi and M. Shimomura, *J. Biomech. Sci. Eng., Special Issue on Micro. Nanobiotech. for Cells,* 2010, **5**, 252.
139 T. Sato, M. Tanaka, S. Yamamoto, E. Ito, K. Shimizu, Y. Igarashi and M. Shimomura, J. Inokuchi, *J. Biomater. Sci. Polym. Ed.,* 2010, **21**, 1947.
140 H. Yabu, M. Takebayashi, M. Tanaka and M. Shimomura, *Langmuir,* 2005, **21**, 3235.
141 M. Tanaka, M. Takebayashi and M. Shimomura, *Macromol. Symp.,* 2009, **279**, 175; M. Tanaka, *Biochimica et Biophysica Acta*, 2011, **1810,** 251.
142 R. J. McMurray, N. Gadegaard, P. M. Tsimbouri, K. V. Burgess, L. E. McNamara, R. Tare, K. Murawski, E. Kingham, R. O. C. Oreffo and M. J. Dalby, *Nat. Mater.,* 2011, **10,** 637.

143 Z. Xiaojun and Z. Shuguang, *Macromol. Biosci.,* 2007, **7**, 13.
144 V. Dinca , E. Kasotakis, J. Catherine, A. Mourka , A. Ranella, A. Ovsianikov, B. N. Chichkov, M. Farsari , A. Mitraki and C. Fotakis, *Nano Letters*, 2007, **8,** 538.
145 E. Gazit, *Chem. Soc. Rev.,* 2007, **36**, 1263.
146 S. Scanlon and A. Aggeli, *Nanotoday,* 2008, **3**, 22.
147 S. Zhang, *Materials Today*, 2003, **6**, 20.
148 J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang and A. J. Grodzinsky, *Proc. Natl. Acad. Sci. USA,* 2002, **99**, 9996.
149 F. Gelain, D. Bottai, A. Vescovi, S. Zhang, *PLoS ONE,* 2006, **1**, e119.
150 S. Zhang, F. Gelain, X. Zhao, *Semin. Cancer Biol*. 2005, **15**, 413.
151 R. G. Ellis-Behnke, Y. X. Liang, S.-W. You, D. K. C. Tay, S. Zhang, K.-F. So, and G. E. Schneider, *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 5054.
152 S. Zhang, T. Holmes, C. Lockshin, A. Rich, *Proc. Natl. Acad. Sci. USA,* 1993, **90**, 3334.
153 T. C. Holmes, S. de Lacalle, X. Su, G. Liu, A. Rich, and S. Zhang, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6728.
154 J. M. Jukes, L. Moroni, C. A. van Blitterswijk and J. de Boer, *Tissue Eng. A,* 2008, **14**, 135.
155 S. Gerecht, J. A. Burdick, L. S. Ferreira, S. A. Townsend, R. Langer and Vunjak-G. Novakovic, *Proc. Natl. Acad. Sci. USA,* 2007, **104**, 11298.
156 J. Elisseeff, A. Ferran, S. Hwang, S. Varghese and Z. Zhang, *Stem Cells Dev.,* 2006, **15,** 295.
157 J. J. Rice, M. M. Martino, .L. D. Laporte, F. Tortelli, P. S. Briqiez and J. A. Hubbell, *Adv. Healthcare Mater.,* 2013, **2***,* 57.
158 J. R. Thonhoff, D. I. Lou, P. M. Jordan, X. Zhao, P. Wu, *Brain Res*., 2008, **1187**, 42.
159 C. Fischbach, R. Chen, T. Matsumoto, T. Schmelzle, J. S. Brugge, P. J. Polverini and D. J. Mooney, *Nat. Methods,* 2007, **4***,* 855.
160 C. Chung and J. A. Burdick, *Adv. Drug Deliv. Rev.,* 2008, **60***,* 243.
161 A. Karkhaneh, Z. Naghizadeh, M. A. Shokrgozar, S. Bonakdar, A. Solouk and N. Haghighipour, *Int. J. Artificial Organs,* 2014, **37**, 142.
162 A. Karkhaneh, Z. Naghizadeh, M. A. Shokrgozar and S. Bonakdar*, J. Appl. Polym. Sci.,* 2014, **131***,* 40635/1.
163 P. M. Favi, R. S. Benson, N. R. Neilsen, R. L. Hammonds, C. C. Bates, C. P. Stephens and M. S. Dhar, *Mater. Sci. Eng. C: Mater. Biolog. Appl.,* 2013, **33***,* 1935.
164 R. L. Mauck, X. Yuan, R. S. Tuan and B. Chief, *Osteoarthritis Cartilage,* 2006, **14***,* 179.
165 C. Chung and J. A. Burdick, *Tissue Eng. Part A,* 2009, **15***,* 243.
166 C. Chung, M. Beecham, R. L. Mauck and J. A. Burdick, *Biomaterials,* 2009, **30**, 4287.
167 Y. H. Jeon, J. H. Choi, J. K. Sung, T. K. Kim, B. C. Cho and H. Y. Chung, *J. Craniofac. Surg.,* 2007, **18**, 1249.
168 Z. Chen, M. Zhao, K. Liu, Y. Wan, X. Li and G. Feng, *J. Mater. Sci.: Mater. Med.* 2014, **25**, 1903.
169 S-.Y. Lee, A-.S. Wee, C-.K. Lim, A. A. Abbas, L. Selvaratnam, A. M. Merican, T. S. Ahmad and T. Kamarul, *J. Mater. Sci.: Mater. Med.,* 2013, **24***,* 1561.
170 C. Fan, L. Liao, C. Zhang and L. Liu, *J. Mater. Chem. B: Mater. Biol. Med.,* 2013, **1***,* 4251.
171 D. A. Rennerfeldt, A. N. Renth, Z. Talata, S. H. Gehrke and M. S. Detamore, *Biomaterials,* 2013, **34**, 8241.
172 F. Yu, X. Cao, L. Zeng, Q. Zhang and X. Chen, *Carbohydr. Polym.,* 2013, **97**, 188.
173 L. R. Almeida, A. R Martins, E. M. Fernandes, M. B. Oliveira, V. M. Correlo, I. Pashkuleva, A. P. Marques, A. S. Ribeiro, N. F. Duraes and C. J. Silva, *Acta Biomater.,* 2013, **9**, 8167.
174 L. D. Solorio, E. L. Vieregge, C. D. Dhami and E. Alsberg, *Tissue Eng. Part B: Reviews,* 2013, **19**, 209.
175 W. Zhao, X. Jin, Y. Cong, Y. Liu and J. Fu, *J. Chemical Technol. Biotechnol.,* 2013, **88,** 327.
176 I. Martin, S. Miot, A. Barbero, M. Jakob and D. Wendt, *J. Biomechanics,* 2007, **40***,* 750.
177 S. R. Frenkel and P. E. Di Cesare, *Ann. Biomedical Eng.* 2004, **32,** 26.
178 Y. Tanaka, H. Yamaoka, S. Nishizawa, S. Nagata, T. Ogasawara, Y. Asawa, Y. Fujihara, T. Takato and K. Hoshi, *Biomaterials* 2010, **31**, 4506.
179 W. Dai, N. Kawazoe, X. Lin, J. Dong and G. Chen, *Biomaterials,* 2010, **31**, 2141.
180 S. J. Shieh, S. Terada and J. P. Vacanti, *Biomaterials* 2004, **25**, 1545.
181 T. B. F. Woodfield, J. Malda, J. de Wijn, F. Peters, J. Riesle and C. A. van Blitterswijk, *Biomaterials,* 2004, **25**, 4149.
182 E. Kon, A. Di Martino, G. Filardo, et al. *Eur. J.Radiology,* 2011, **79**, 382.
183 C. He, W. Nie and W. Feng, *J. Mater. Chem. B,* 2014, **2**, 7828; J. A. Goding, A. D. Gilmour, P. J. Martens, L. A. Poole-Warren and R. A. Green, *J. Mater. Chem. B*, 2015, **3***,* 5058.
184 J. Iwasa, L. Engebretsen, Y. Shima and M. Ochi, *Knee Surg. Sports Traumatol. Arthrosc.,* 2009, **17***,* 561.
185 M. Marcacci, E. Kon, S. Zaffagnini, G. Filardo, M. Delcogliano, M. P. Neri, F. Iacono and A. P. Hollander, *Knee Surg. Sports Traumatol. Arthrosc.,* 2007, **15,** 610.
186 M. Marcacci, S. Zaffagnini, E. Kon, A. Visani, F. Iacono and I. Loreti, *Knee Surg. Sports Traumatol. Arthrosc.,* 2002, **10**, 154.
187 L. E. Niklason, *Nat. Biotechnol.,* 2000, **18**, 929.
188 A. U. Daniels, K. P. Andriano, W. P. Smutz, M. K. O. Chang and J. Heller, *J. Appl. Biomater.,* 1994, **5**, 51.
189 A. J. Thornton, E. Alsberg, M. Albertelli and D. J. Mooney, *Transplantation,* 2004, **77***,* 1798.
190 Z. Li, H. R. Ramay, K. D. Hauch, D. Xiao and M. Zhang, *Biomaterials,* 2005, **26***,* 3919.
191 A. Tampieri, M. Sandri, E. Landi, G. Celotti, N. Roveri, M. Mattioli-Belmonte, L. Virgili, F. Gabbanelli and G. Biagini, *Acta Biomater.,* 2005, **1,** 343.
192 Y. Khan, M. J. Yaszemski, A. G. Mikos and C. T. Laurencin, *J. Bone Joint Surg. Am.,* 2008, **90**, 36.
193 A. Hussein, A. Nadia and A. K. Numa, *Res. J. Pharm. Biol. Chem. Sci.,* 2014, **5**, 295.
194 N. Sultana, *Appl. Mech. Mater.,* 2014, **554**, 42.
195 Y. Hu, X. Gu, Y. Yang, J. Huang, M. Hu, W. Chen, Z. Tong and C. Wang, *ACS Appl. Mater. Interfaces,* 2014, **6**, 17166.
196 H. Y. Mi, S. M. Palumbo, X. Jing, L. S. Turng, W. J. Li and X. F. Peng, *J. Biomed. Mater. Res. Part B: Appl. Biomater.*, 2014, **102B**, 1434.
197 J. Minton, C. Janney, R. Akbarzadeh, C. Focke, A. Subramanian, T. Smith, J. McKinney, J. Liu, J. Schmitz and P. F. James, *J. Biomater. Sci., Polym. Ed.,* 2014, **25**, 1856.
198 A. R. Costa-Pinto, R. L. Reis and N. M. Neves, *Tissue Eng., Part B: Rev.,* 2011, **17***,* 331.
199 W. B. Tsai, Y. R. Chen, H. L. Liu and J. Y. Lai, *Carbohyd. Polym.,* 2011, **85**, 129.
200 A. M. Martins, C. M. Alves, K. F. Kurtis A. G. Mikos and R. L. Reis, *J. Mater. Chem.,* 2010, **20**, 1638.
201 A. R. Costa-Pinto, V. M. Correlo, P.C. Sol, M. Bhattacharya, P. Charbord, B. Delorme, R. L. Reis and N. M. Neves, *Biomacromolecules,* 2009, **10***,* 2067.
202 M. S. Bae, D. H. Yang, J. B. Lee, D. N. Heo, Y. D. Kwon, I. C. Youn, K. Choi, J. H. Hong, G. T. Kim and Y. S. Choi, *Biomaterials,* 2011, **32**, 8161.
203 W. R. Walsh, R. M. Oliver, G. Gage, Y. Yu, D. Bell, J. Bellemore and H. D. Adkisson, *Tissue Engin. Part A,* 2011, **17**, 213.
204 T. B. L. Nguyen, Y. K. Min and B. T. Lee, *J. Mater. Sci.,* 2013, **48***,* 4233.

205 G. Hulsart-Billstroem, P. K. Yuen, R. Marsell, J. Hilborn, S. Larsson and D. Ossipov, *Biomacromolecules,* 2013, **14**, 3055.
206 F. D. Ivan, A. Marian, C. E. Tanase, M. Butnaru and L. Verestiuc, *Key Eng. Mater.,* 2014, **587**, 191.
207 M. Neufurth, X. Wang, H. C. Schroeder, Q. Feng, B. Diehl-Seifert, T. Ziebart, R. Steffen, S. Wang and W. E. G. Mueller, *Biomaterials,* 2014, **35**, 8810.
208 A. Moshaverinia, C. Chen, X. Xu, K. Akiyama, S. Ansari, H. H. Zadeh, Homayoun and S. Shi, *Tissue Eng. Part A,* 2014, **20***,* 611.
209 R. Nakaoka, Y. Hirano, D. J. Mooney, T. Tsuchiya and A. Matsuoka, *J. Artif. Organs,* 2013, **16***,* 284.
210 A. Moshaverinia, S. Ansari, C. Chen, X. Xu, K. Akiyama, M. L. Snead, H. H. Zadeh and S. Shi, *Biomaterials,* 2013, **34***,* 6572.
211 N. Shanmugasundaram, P. Ravichandran, P. Neelakanta Reddy, N. Ramamurty, S. Pal and K. P. Rao, *Biomaterials,* 2001, **22***,* 1943.
212 J. K. Park, J. H. Shim, K. S. Kang, J. Yeom, H. S. Jung, J. Y. Kim, K. H. Lee, T. H. Kim, S. Y. Kim and D. W. Cho, *Adv. Funt. Mater.,* 2011, **21***,* 2906.
213 F. Khan and S. R. Ahmad, *Macomol. Biosci.,* 2013, **13**, 395.
214 N. Siddiqui and K. Pramanik, *J. Appl. Polym. Sci.,* 2014, **131***,* 41025/1.
215 S. Mathews, R. Bhonde, P. K. Gupta and S. Totey, *J. Biomed. Mater. Rese. Part B: Appl. Biomater.,* 2014, **102***,* 1825.
216 T. B. L. Nguyen, Y. K. Min and B. T. Lee, *J. Mater. Sci.,* 2013, **48,** 4233.
217 J. H. Park, E. J. Lee, J. C. Knowles and H. W. Kim, *J. Biomater. Appl.,* 2014, **28***,* 1079.
218 R. A. Perez, M. Kim, T. H. Kim, J. H. Kim, J. H. Lee, J. H. Park, J.C. Knowles and H. W. Kim, *Tissue Eng. Part A,* 2014, **20***,* 103.
219 N. Thadavirul, P. Pavasant and P. Supaphol, *J. Biomed. Mater. Res. Part A,* 2014, **102A***,* 3379.
220 J. Lim, M. S. K. Chong, J. K. Y. Chan and S. H. Teoh, *Small,* 2014, **10***,* 2495.
221 G. Z. Jin, T. H. Kim, J. H. Kim, J. E. Won, S. Y. Yoo, S. J. Choi, J. K. Hyun and H. W. Kim, *J. Biomed. Mater. Res. Part A,* 2013, **101A***,* 1283.
222 T. Jacobs, H. Declercq, N. Geyter, R. Cornelissen, P. Dubruel, C. Leys, A. Beaurain, E. Payen and R. Morent, *J. Mater. Sci.: Mater. Med.,* 2013, **24***,* 469.
223 H. Mahjoubi, J. M. Kinsella, M. Murshed and M. Cerruti, *ACS Appl. Mater. Interfaces,* 2014, **6,** 9975.
224 A. Das, S. Tanner, D. A. Barker, D. Green, E. A. Botchwey, *J. Biomed. Mater. Res. Part A,* 2014, **102A***,* 1210.
225 M. Bao, X. Lou, Q. Zhou, W. Dong, H. Yuan and Y. Zhang, *ACS Appl. Mater. Interfaces,* 2014, **6***,* 2611.
226 A. Sadiasa, T. H. Nguyen and B. T. Lee, *J. Biomater. Sci. Polym. Ed.,* 2014, **25***,* 150.
227 E. Ko, K. Yang, J. Shin and S-.W. Cho, *Biomacromolecules,* 2013, **14***,* 3202.
228 N. Y. C. Yu, A. Schindeler, L. Peacock, K. Mikulec, J. Fitzpatrick, A. J. Ruys, J. J. Cooper-White and D. G. Little, *Eur. Cells Mater.,* 2013, **25***,* 190.
229 C. Zhou, Q. Shi, W. Guo, L. Terrell, A. T. Qureshi, D. J. Hayes and Q. Wu, *ACS Appl. Mater. Interfaces,* 2013, **5***,* 3847.
230 B. Torabinejad, J. Mohammadi-Rovshandeh, S. M. Davachi and A. Zamanian, *Mater. Sci. Eng. C: Mater. Biol. Appl.,* 2014, **42***,* 199.
231 T. Lou, X. Wang, G. Song, Z. Gu and Z. Yang, *Int. J. Biol. Macromol.,* 2014, **69**, 464.
232 J. M. Fernandez, M. S. Cortizo and A. M. Cortizo, *J. Biomater. Tissue Eng.,* 2014, **4***,* 227.
233 X. Zhang, W. Chang, P. Lee, Y. Wang, M. Yang, J. Li, S. G. Kumbar and X. Yu, *PLoS One,* 2014, **9**, e85871/1.
234 C. Zong, X. Qian, Z. Tang, Q. Hu, J. Chen, C. Gao, R. Tang, X. Tong and J. Wang, *J. Biomed. Nanotech.,* 2014, **10***,* 1091.
235 W. Lu, K. Ji, J. Kirkham, Y. Yan, A. P. Boccaccini, M. Kellett, Y. Jin and X. B. Yang, *Cell Tissue Res.,* 2014, **356**, 97.
236 N. Sultana and T. H. Khan, *J. Bionanoscience,* 2013, **7**, 169.
237 K. R. Remya, J. Joseph, S. Mani, A. John, H. K. Varma and P. Ramesh, *J. Biomed. Nanotech.,* 2013, **9***,* 1483.
238 W. Chang, X. Mu, X. Zhu, G. Ma, C. Li, F. Xu and J. Nie, *Mater. Sci. Eng. C: Mater. Biol. Appl.,* 2013, **33***,* 4369.
239 A. B. Kutikov and J. Song, *Acta Biomater.,* 2013, **9**, 8354.
240 W. Y. Choi, H .E. Kim and Y. H. Koh, *J. Porous Mater.,* 2013, **20***,* 701.
241 C. Gao, B. Yang, H. Hu, J. Liu, C. Shuai and S. Peng, *Mater. Sci. Eng. C: Mater. Biol. Appl.,* 2013, **33***,* 3802.
242 M-.J.Chern, Y.-K. Shen and H.-H. Hung, *Adv. Sci. Lett.,* 2013, **19***,* 2572.
243 F. Khan and S. R. Ahmad, in *Biomimetics: Advancing Nanobiomaterials and Tissue Engineering* (Eds: M. Ramalingam, X. Wang, G. Chen, P. Ma and F. Z. Cui), Scrivener Publishing, WILEY **2013**, Ch.5, pp 91-117.
244 R. A. Thibault, A. G. Mikos and F. K. Kasper, *Adv. Healthcare Mater.,* 2013, **2***,* 13.
245 L. B. Rocha, G. Goissis and M. A. Rossi, *Biomaterials,* 2002, **23***,* 449.
246 L. S. Liu, A. Y. Thompson, M. A. Heidaran, J. W. Poser and R. C. Spiro, *Biomaterials,* 1999, **20**, 1097.
247 S. H. Oh, S. G. Kang, E. S. Kim, S. H. Cho and J. H. Lee, *Biomaterials,* 2003, **24***,* 4011.
248 D. J. Trantolo, S. T. Sonis, B. M. Thompson, D. L. Wise, K. U. Lewandrowski and D. D. Hile, *Int. J. Oral. Maxillofac. Implants,* 2003, **18***,* 182.
249 M. Bradley, F. Khan, R. O. C. Oreffo and R. S. Tare, WO 2010/021443 A2.
250 J. O. Smith, E. R.Tayton, F. Khan, A. Aarvold, R. B. Cook, A. Goodship, M. Bradley and R. O. C. Oreffo. *J. Tissue Eng. Regen. Med.,* 2014, *In Press.* DOI: 10.1002/term.2007.
251 E. C. Tsai, P. D. Dalton, M. S. Shoichet and C. H. Tator, *Biomaterials,* 2006, **27**, 519.
252 M. Dadsetan, A. M. Knight, L. Lu, A. J. Windebank and M. J. Yaszemski, *Biomaterials,* 2009, **30**, 3874.
253 A. Hejcl, P. Lesny, M. Pradny, J. Sedy, J. Zamecnik, P. Jendelova, J. Michalek and E. Sykova, *J. Mater. Sci.: Mater. Med.,* 2009, **20**, 1571.
254 S. R. Hynes, M. F. Rauch, J. P. Bertram and E. B. Lavik, *J. Biomed. Mater. Res.,* 2009**, 89A**, 499.
255 C. Y. Yang, B. Song, Y. Ao, A. P. Nowak, R. B. Abelowitz, R. A. Korsak, L. A. Havton, T. J. Deming and M. V. Sofroniew, *Biomaterials,* 2009, **30**, 851.
256 D. R. Nisbet, D. Moses, T. R. Gengenbach, J. S. Forsythe, D. I. Finkelstein and M. K. Horne, *J. Biomed. Mater. Res.,* 2009**, 89A***,* 24.
257 P. Krsko, T. E. McCann, T. T. Thach, T. L. Laabs, H. M. Geller and M. R. Libera, *Biomaterials,* 2009, **30**, 721.
258 D. R. Nisbet, K. E. Crompton, M. K. Horne, D. I. Finkelstein and J. S. Forsythe, *J. Biomed. Mater. Res. Part B: Appl. Biomater.,* 2008, **87B**, 251.
259 M. C. Dodla and R. V. Bellamkonda, *Biomaterials,* 2008, **29**, 33.
260 R. Balint, N. J. Cassidy and S. H. Cartmell, *Acta Biomater.,* 2014, **10***,* 2341.
261 H. Xu, J. M. Holzwarth, Y. Yan, P. Xu, H. Zheng, Y. Yin, S. Li and P. X. Ma, *Biomaterials,* 2014, **35***,* 225.
262 J. E. Collazos-Castro, G. R. Hernandez-Labrado, J. L. Polo and C. Garcia-Rama, *Biomaterials,* 2013, **34***,* 3603.
263 Z. Zhou, P. Yu, H. M. Geller and C. K. Ober, *Biomacromolecules,* 2013, **14***,* 529.
264 P. Kumar, Y. E. Choonara, L. C. du Toit, G. Modi, D. Naidoo and V. Pillay, *Int. J. Molecul. Sci.,* 2012, **13**, 13966.
265 S. Guan, X. L. Zhang, X. M. Lin, T. Q. Liu, X. H. Ma and Z. F. Cui, *J. Biomater. Sci. Polym. Ed.,* 2013, **24***,* 999.

266 J. F. Cherry, A. L. Carlson, F. L. Benarba, S. D. Sommerfeld, D. Verma, G. Loers, J. Kohn, M. Schachner and P. V. Moghe, *Biointerphases,* 2012, **7**, 22.

267 S. N. Alhosseini, F. Moztarzadeh, M. Mozafari, S. Asgari, M. Dodel, A. Samadikuchaksaraei, S. Kargozar and N. Jalali, *Int. J. Nanomed.,* 2012**, 7**, 25.

268 M. P. Prabhakaran, L. Ghasemi-Mobarakeh, G. Jin and S. Ramakrishna, *J. Biosci. Bioeng.,* 2011**, 112**, 501.

269 Y.-S. Lee, G. Collins and A. T. Livingston, *Acta Biomater.,* 2011, **7**, 3877.

270 L. Ghasemi-Mobarakeh, M. P. Prabhakaran, M. Morshed, M. H. Nasr-Esfahani, H. Baharvand, S. Kiani, S. S. Al-Deyab and S. Ramakrishna, *J. Tissue Eng. Regen. Med.,* 2011**, 5(4)**, e17.

271 V. Lundin, A. Herland, M. Berggren, E. W. H. Jager and A. I. Teixeira, *PLoS One,* 2011, **6(4),** e18624.

272 Q. Tu, L. Li, Y. Zhang, J. Wang, R. Liu, M. Li, W. Liu, X. Wang, L. Ren and J. Wang, *Biomaterials,* 2011, **32,** 3253.

273 S. H. Ku, M. Lee and C. B. Park, *Adv. Healthcare Mater.*, 2013, **2,** 244.

274 X. Luo, C. L. Weaver, S. Tan and X. T. Cui, *J. Mater. Chem. B: Mater. Biol. Med.,* 2013, **1,** 1340.

275 K. Zhou, G. A. Thouas, C. C. Bernard, D. R. Nisbet, D. I. Finkelstein, D. Li and J. S. Forsythe, *ACS Appl. Mater. Interfaces,* 2012, **4**, 4524.

276 G. Z. Jin, M. Kim, U. S. Shin, H. W. Kim, *Neurosci. Lett*., 2011, **501,** 10.

277 G. Z. Jin, M. Kim, U. S. Shin and H. W. Kim, *Cell Biol. Int.,* 2011**, 35**, 741.

278 J. Ai, A. Kiasat-Dolatabadi, S. Ebrahimi-Barough, A. Ai, N. Lotfibakhshaiesh, A. Norouzi-Javidan, H. Saberi, B. Arjmand, H. R. Aghayan, *Arch. Neurosci.*, 2014, **1,** 15.

279 T. Freier, R. Montenegro, H. Shan Koh, M. S. Shoichet, *Biomaterials,* 2005, **26,** 4624.

280 S. B. Lee, Y. H. Kim, M. S. Chong, S. H. Hong and Y. M. Lee, *Biomaterials,* 2005, **26**, 1961.

281 Z. A. A. Hamid, A. Blencowe, B. Ozcelik, et al. *Biomaterials,* 2010, **31**, 6454.

282 H. Fan, H. Liu, L. T. Siew and J. C. H. Goh, *Biomaterials,* 2009, **30**, 4967.

283 K. Lee, E. A. Silva and D. J. Mooney, *J. R. Soc. Interface,* 2011, **8,** 153.

284 K. Hori, C. Sotozono, J. Hamuro, K. Yamasaki, Y. Kimura, M. Ozeki, Y. Tabata and S. Kinoshita, *J. Controlled Release,* 2007, **118**, 169.

285 T. Wang, X. Jiang, T. Lin, S. Ren, X. Li, X. Zhang and Q. Tang, *Biomaterials,* 2009, **30**, 4161.

286 D. M. Yoon, E. C. Hawkins, S. Francke-Carroll and J. P. Fisher, *Biomaterials,* 2007, **28**, 299.

287 L. R. Rodino-Klapac, A. M. Haidet, J. Kota, C. Handy, B. K. Kaspar and J. R. Mendell, *Muscle Nerve,* 2009, **39**, 283.

288 S. J. Jhaveri, M. R. Hynd, N. Dowell-Mesfin, J. N. Turner, W. Shain and C. K. Ober, *Biomacromolecules,* 2009, **10**, 174.

289 L. W. Hosack, M. A. Firpo, J. A. Scott, G. D. Prestwich and R. A. Peattie, *Biomaterials,* 2008, **29**, 2336.

290 B. Sullenbarger, J. H. Bahng, R. Gruner, N. Kotov and L. C. Lasky, *Experimental Hematology,* 2009, **37**, 101.

291 X. Guo, H. Park, G. Liu, W. Liu, Y. Cao, Y. Tabata, F. K. Kasper and A. G. Mikos, *Biomaterials,* 2009, **30**, 2741.

292 J. Yu, Y. Gu, K. T. Du, S. Mihardja, R. E. Sievers and R. J. Lee, *Biomaterials,* 2009, **30**, 751.

293 S. B. Lowe, V. T. G. Tan, A. H. Soeriyadi, T. P. Davies and J. J. Gooding, *Bioconjugate Chem.,* 2014, **25(9),** 1581.

294 N. Yonet-Tanyeri, M. H. Rich, M. Lee, M.-H. Lai, J. H. Jeong, R. J. DeVolder and H. Kong, *Biomaterials,* 2013, **34,** 8416.

295 S. P. Herbert and D. Y. Stainier, *Nat. Rev. Mol. Cell Biol.,* 2011**, 12**, 551.

296 O. P. Gautschi, S. P. Frey and R. Zellweger, *ANZ J. Surg.,* 2007, **77**, 626.

297 M. K. Nagai and J. M. Embil, *Expert Opin. Biol. Ther.,* 2002, **2**, 211 .

298 I. J. Frieden, *Pediatr. Dermatol.,* 2008, **25**, 590.

299 E. J. Carragee, E. L. Hurwitz and B. K. Weiner, *Spine J.,* 2011, **11**, 471.

300 K. R. Garrison, S. Donell, J. Ryder, I. Shemilt, M. Mugford, I. Harvey and F. Song, *Health Technol. Assess.,* 2007, **11**, 1.

301 D. H. R. Kempen, M. C. Kruyt, L. Lu, C. E. Wilson, A. V. Florschutz, L. B. Creemers, M. J. Yaszemski and W. J. A. Dhert, *Tissue Eng. Part A,* 2009, **15**, 587.

302 H. Park, J. S. Temenoff, Y. Tabata, A. I. Caplan, R. M. Raphael, J. A. Jansen and A. G. Mikos, *J. Biomed. Mater. Res.,* 2009, **88A**, 889.

303 R. Baffour, J. L. Garb, J. Kaufman, J. Berman, S. W. Rhee, M. A. Norris and P. Friedmann, *J. Surg. Res.,* 2000, **93**, 219.

304 S. Nakamura, M. Ishihara, K. Obara, K. Masuoka, T. Ishizuka, Y. Kanatani, B. Takase, T. Matsui, H. Hattori, T. Sato, Y. Kariya and T. Maehara, *J. Biomed. Mater. Res.,* 2006, **78A**, 364.

305 J. J. Yoon, H. J. Chung and T. G. Park, *J. Biomed. Mater. Res.,* 2007, **83A**, 597.

306 Y. Liu, L. Sun, Y. Huan and H. Zhao, J. Deng, *J. Surg. Res.,* 2006, **136**, 85.

307 L. Chen, W. Jiang, J. Huang, B. C. He, G. W. Zuo, W. Zhang, et al, *J. Bone Mineral Res.,* 2010, **25**, 2447.

308 B. Wolach, L. J. van der Laan, N. A. Maianski, T. A. Tool, R. van Bruggen, D. Roos and T. W. Kuijpers, *Exp. Hematol.,* 2007 **35(4),** 541.

309 Q. Zeng, X. Li, G. Beck, G. Balian and F. H. Shen, *Bone,* 2007**, 40**, 374.

310 A. C. McPherron, A. M. Lawler and S. J. Lee, *Nature,* 1997, **387**, 83.

311 J. R. Silva, R. van den Hurk, H. T. van Tol, B. A. Roelen, J. R. Figueiredo, *Mol. Reprod. Dev.,* 2005, **70**, 11.

312 N.S. Cunningham, N.A Jenkins, D.J. Gilbert, N. G. Copeland, A. H. Reddi and S. J. Lee, *Growth Factors,* 1995, **12,** 99.

313 R. E. Andersen and D. A. Lim, *Cell Research*, 2014, **24**, 1381.

314 E. Schmelzer, A. Deiwick, H. Bruns, H. C. Fiegel and A. Bader, *Eur. J. Gastroenterol. Hepatol.***,** 2008, **20,** 209.

Fabrication of Polymeric Biomaterials: A Strategy for Tissue Engineering and Medical devices

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Keyword: Polymeric Biomaterials, Scaffolds, Hydrogels, Tissue Engineering, Cartilage, Bone and Neural Tissue.

Fabrication of biomaterials scaffolds using various methods and techniques are discuused, utilising natural and synthetic polymers and their composites, for tissue regeneration and medical devices applications. This review covers the various advanced methods for 3D structural scaffolds fabrication, self-assembled micro-/nano-structure scaffolds generation and hydrogels with and without growth factors, for cell adhesion, proliferation, and/or differentiation, and for ultimate tissue regeneration.