Chapter 11
Photofablication Techniques for 3D Tissue Construct

Shinji Sugiura, Fumiki Yanagawa and Toshiyuki Kanamori

Abstract Living cells in human body exhibit their function in microstructured three dimensional (3D) environments composed of soluble factors, extracellular matrix (ECM), and neighboring cells. Fabricating biological 3D *in vitro* models of tissue and organ is a critical step for developing new strategies for cell based assay in drug discovery. To date, a number of research groups have developed micropatterning of hydrogels using photocurable and photodegradable hydrogels. This chapter reviews recent development on the techniques for microscale 3D fabrication of biomaterials by means of photolithography. Also, this chapter introduces an activated-ester-type photocleavable crosslinker, which we recently synthesized to generate photodegradable hydrogels using biocompatible materials such as polyethylene glycol and gelatin. This new type of crosslinker enabled convenient preparation of photodegradable hydrogel by two component mixing reaction. The hydrogels were degraded by micropatterned light irradiation, local light irradiation, and two-photon excitation. This simple and convenient approach to prepare and fabricate photodegradable hydrogels is creating new opportunity for novel cell manipulation and 3D tissue engineering techniques.

Keywords Tissue engineering · Light · Hydrogel · Biomaterials · Photodegradation

11.1 Introduction

In order to develop new cell based assay technique for drug discovery, researchers in academia and industry require *in vitro* models that could reproduce biological events *in vivo* models. Current two dimensional (2D) *in vitro* platforms are useful for investigating the molecular responses in tissue and organ based on physiological and pathological studies. Recent advances in molecular biology can identify the proteins, receptors and ligands responses 2D *in vitro* models. However, these

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2D *in vitro* platforms do not precisely simulate the complicated cell-cell and cell-extracellular matrix (ECM) interactions, while cells *in vivo* communicate with each other in a microstructured three dimensional (3D) environment in response to soluble factors, ECM molecules and intercellular contact dependent signals. Therefore, cell culture on 2D substrates, which has been employed in conventional biological research, does not adequately recapitulate the 3D nature of native cellular microenvironment [1, 2]. In addition, spatially uniform and static materials lack the intricate spatial and temporal aspects of *in vivo* systems. Cells dynamically respond to the local microenvironment during diverse processes such as tissue morphogenesis, stem cell differentiation, cancer progression [3, 4]. Recapitulating such dynamic microenvironments 3D *in vitro* platforms would have high potential impact in cell biology by providing an excellent model for systematic differentiation of stem cells, and for understanding of tissue regeneration, ultimately leading to more rational tissue engineering strategies. Recently, the approaches for fabricating 3D *in vitro* models using biocompatible hydrogels based on top-down and bottom-up methods have been used in stem cell research, regenerative medicine and tissue engineering. In particular, photoresponsible hydrogels such as photocurable and photodegradable hydrogels have been employed to reproduce complicated structures for mimicking complicated 3D tissue and organ.

11.2 Photocurable Hydrogels

Photocrosslinkable polymers are popular materials for controlling not only hydrogel microstructure and stiffness but also cellular behavior on and within the hydrogels. A number of research groups have generated micropatterned hydrogels for tissue engineering applications with photocrosslinked polymers, including derivatives of poly(ethylene glycol) diacrylate (PEGDA) [5–7], polysaccharides [8–10], and proteins (e.g., gelatin) [11, 12]. In order to construct 3D Microarchitecture, photolithography and stereolithography have been applied to fabricate hydrogels using these photocurable materials [12, 13–17, 18]. Also, the approaches can provide the 3D *in vitro* platforms for controlling systematic differentiation of stem cells [13, 19]. Although Photocurable Hydrogels are useful materials to construct complex engineered tissues, they may not be readily applicable to generating complex 3D vascularized tissue. Eventually, either microengineered 3D porous or perfusable structures in these hydrogels to perform long-term cell culture are necessary for supplying oxygen, nutrients and other soluble factors for cell growth inside engineered 3D thick tissues [7, 12, 13, 15, 16, 17, 18, 20]. To construct perfusable 3D thick tissue, fabrication of porous or hollow structure in hydrogels is desirable. For this application, use of photodegradable hydrogels, which are degraded by light irradiation, can be another possible approach.

11.3 Photodegradable Hydrogels

Recently, photodegradable hydrogels have attracted significant attention due to their tunable mechanical, chemical properties and their use in the fabrication of 3D microstructures [21, 22] in biomaterials and tissue engineering research [23]. Such photodegradable hydrogels have garnered substantial attention from the biomaterials and tissue engineering research fields [24, 25]. The physical and chemical properties of photodegradable hydrogels can be both temporally and spatially controlled by irradiation with light (single- and two-photon) [26, 27], and this process is compatible with living cells [21, 28].

To date, despite reporting many approaches, current methods to prepare photodegradable hydrogels are restricted to reactions between synthetic molecules, such as radical reactions [21, 27, 29, 30–32], Michael-type conjugations [33], and orthogonal click reactions [22, 34]. In these reactions, one class of convenient hydrogel preparation methods is multicomponent mixing reaction, in which two or more molecular-scale components react with each other and gelation takes place spontaneously after mixing. Such systems are highly tunable as either component can be easily modified to alter the hydrogel performance or to introduce additional functionalities. In the previous study, Michael-type conjugations [34] and orthogonal click reactions [22, 33] have been applied to fabrication of photodegradable hydrogels, by multicomponent mixing reaction. However, these reactions require chemical modification of the component materials. In addition, most of the resulting photodegradable hydrogels were composed of derivatives of PEG or polysaccharides [22, 34], which are biologically inactive; cells can neither bind to nor degrade them. This lack of cell-responsive features possibly affects the viability and activities of cells, and greatly limits the cultural types of cells and applicable culture conditions. Although cell-binding sequences such as Arg-Gly-Asp and other peptides can be incorporated into photodegradable hydrogels by using a combination of synthetic homo- and heterofunctional crosslinkers [21, 22], development of simple systems to prepare biologically functional photodegradable hydrogels can facilitate widespread use of this technology.

11.4 Activated-Ester-Type Photocleavable Crosslinker

We recently reported an activated-ester-type photocleavable crosslinker, namely photocleavable *N*-hydroxysuccinimide (NHS) tetra-arm polyethylene glycol (NHS-PC-4armPEG), which facilitated the formation of photodegradable hydrogels by means of a one-step, two-component mixing reaction with a biocompatible polymer containing amino moieties (amino-terminated tetra-arm PEG (amino-4armPEG) or gelatin) [35]. The NHS-PC-4armPEG is composed of the following functional groups (Fig. 11.1): (i) poly(ethylene glycol) as a water-soluble main polymer chain; (ii) nitrobenzyl groups, which are cleavable in response to light irradiation; and

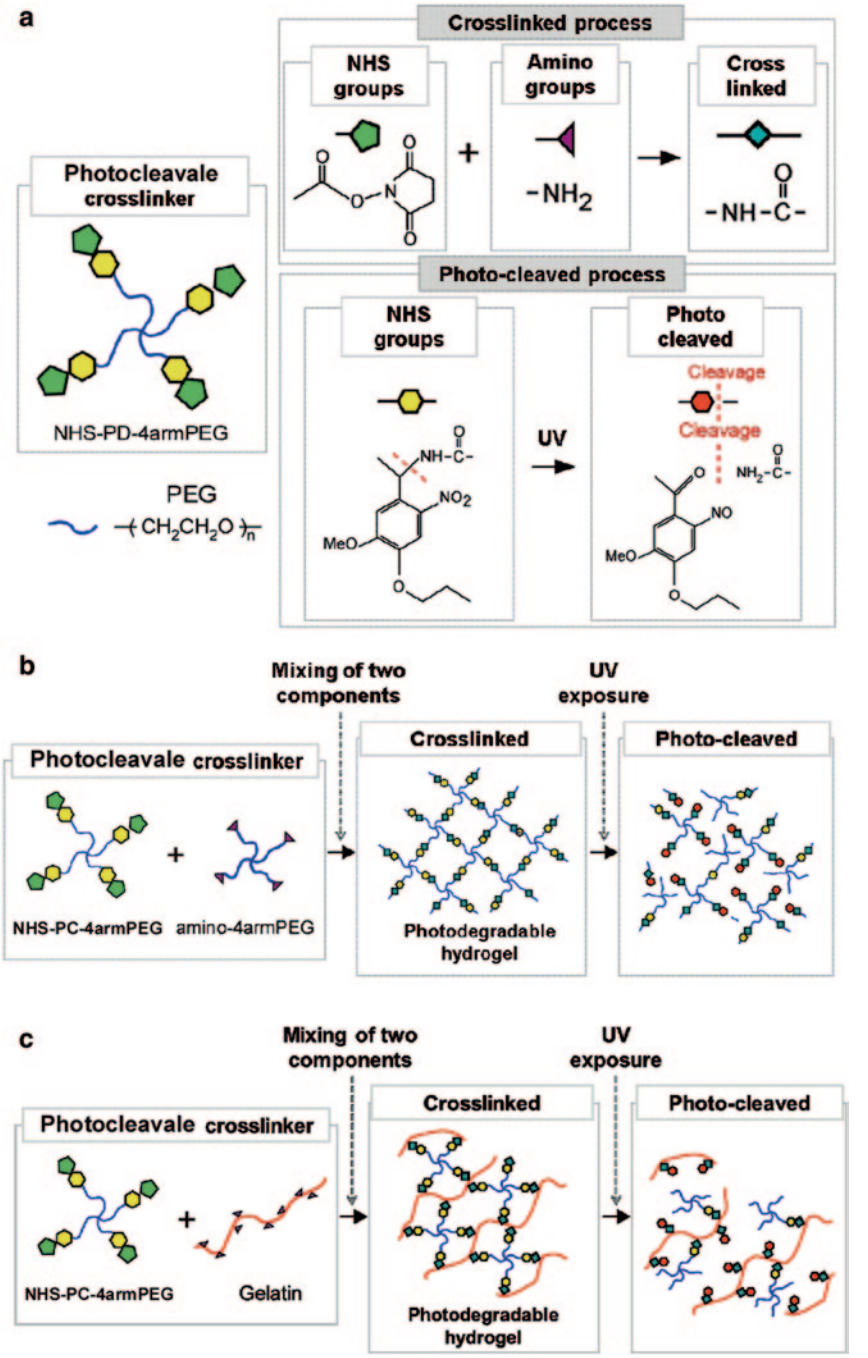

Fig. 11.1 Shematic diagram of, **a** chemical structure and cleavage process of photocleavable crosslinker. Shematic diagram of degradation process of photo-degradable hydrogels with, **b** amino-4arm PEG and, **c** gelatin

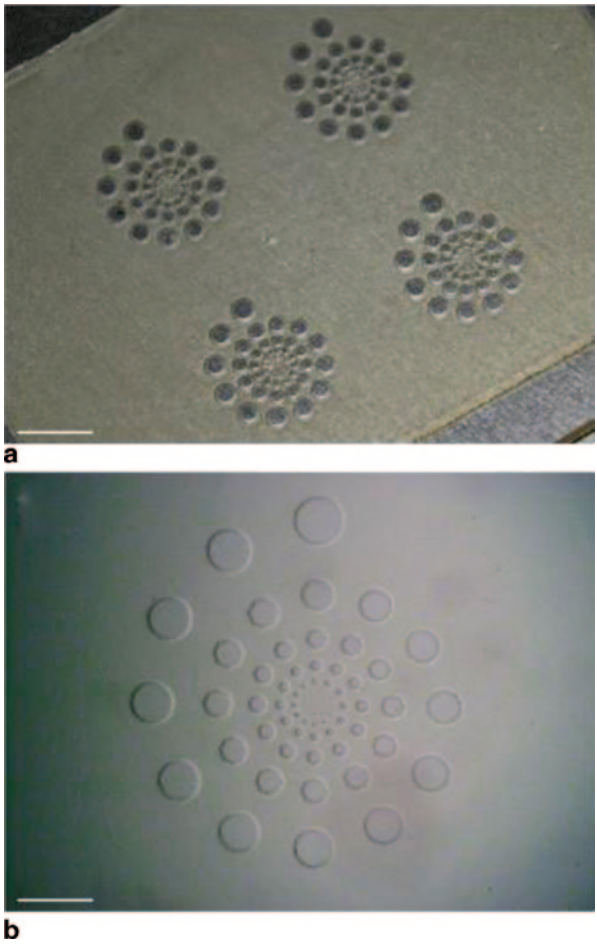

Fig. 11.2 Microscopic images of patterned photodegradable hydrogels crosslinked with amino-4arm PEG (**a**), and gelatin (**b**). Bars = 1 mm (**a**) and 500 μm (**b**)

(iii) NHS ether groups, which react with primary amine groups and form peptide bonds under physiological conditions.

To demonstrate hydrogel micropatterning by photolithography, prepolymer solution which included 5.0 mM amino-4arm polyethylene glycol (amino-4arm-PEG, Mw = 9,617) or 2.5 % (w/v) gelatin was mixed with 0.25–5.0 mM NHS-PC-4armPEG solution. After mixing of the two components, a drop of mixture was placed onto an amino coated glass slide covered by a cover slip and separated by a cover slip or pet film spacers to provide hydrogel units with controlled thicknesses. Subsequently, film masks were placed on a cover slip between light source and hydrogel, and then each sample were exposed to light. Light irradiation through film mask degraded exposed regions in hydrogels and created micropatterned structure (Fig. 11.2), whereas unexposed regions remained intact. Patterning on

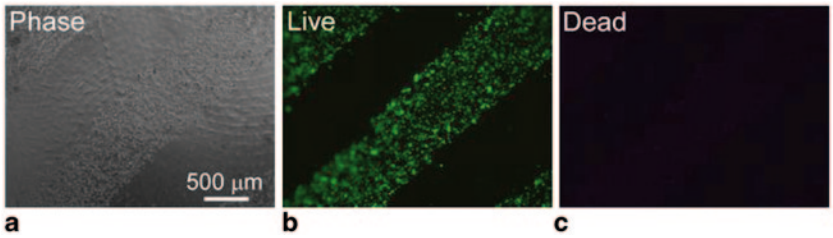

Fig. 11.3 Cell encapsulation (HepG2) in patterned photodegradable hydrogels prepared with gelatin and photocleavable crosslinker. Live/dead test was carried out after 2 days of culture.

photodegradable hydrogels with microscale precision (20–500 μm) by photolithography was achieved. To estimate morphology of hydrogels degradation, microparticles suspension was put onto patterned hydrogels and the 3D images were captured using confocal microscope. As expected, the depth of degraded regions increased proportionally to light exposure time. That is, morphology of degraded regions was a function of the amount of light exposure energy. We have also demonstrated cell micropatterning in the photodegradable hydrogel (Fig. 11.3). Human liver carcinoma (HepG2) cells has been successfully encapsulated in gelatin based photodegradable hydrogel and patterned by micropatterned light irradiation with maintaining their viability.

11.5 Conclusion

Our new Photocleavable crosslinker can be utilized to prepare photodegradable hydrogel using biomaterials by two-component mixing. Theoretically, NHS group can crosslink any molecules with primary amine. Therefore, many types of components including synthetic polymer, peptide, and cells can be crosslinked in the photodegradable hydrogels (Fig. 11.4). The prepared photodegradable hydrogel were precisely fabricated with desired morphology by micropatterned light irradiation through photomask, local light irradiation from maskless light irradiation system, and two-photon reaction. We recently applied our photodegradable hydrogel and maskless light irradiation system to single cell manipulation in the hydrogels [36]. These approaches could provide variety of tools to fabrication, manipulation, and evaluation of cells and 3D tissues in the future.

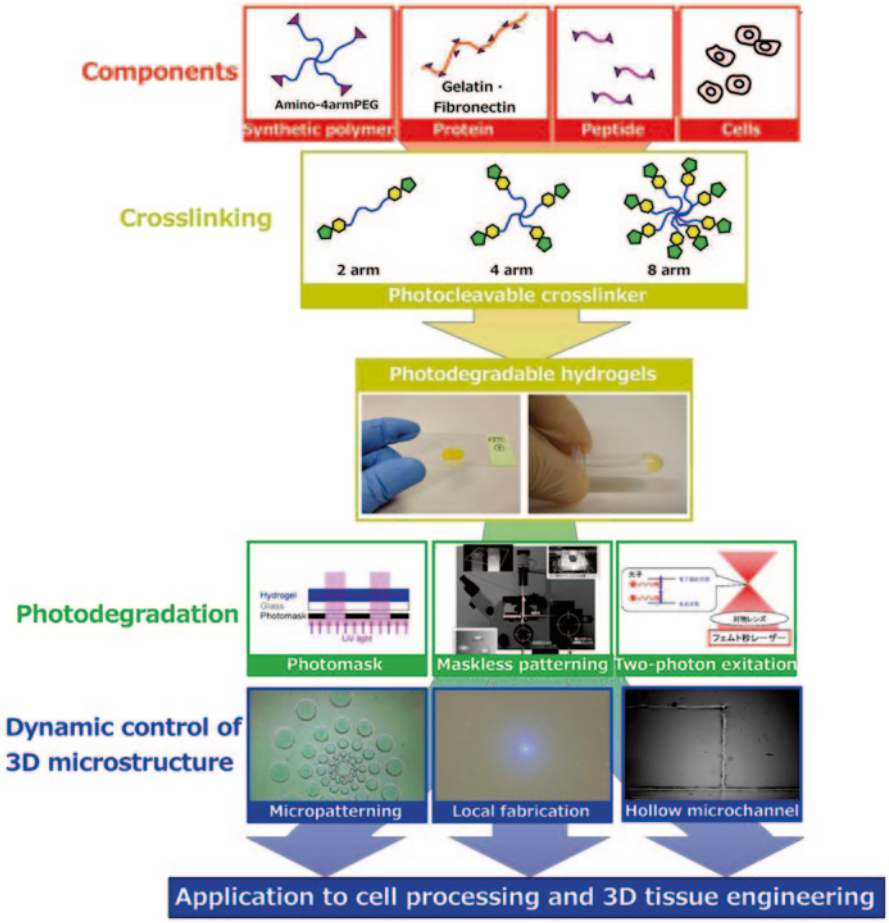

Fig. 11.4 Potential application of photodegradable hydrogels

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