Chapter 23
Tough Double-Network Hydrogels as Scaffolds for Tissue Engineering:
Cell Behavior *in vitro* and *in vivo* Test

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ABSTRACT

Hydrogels are used as scaffolds for tissue engineering in vitro & in vivo because their three-dimensional network structure and viscoelasticity are similar to those of the macromolecular-based extracellular matrix (ECM) in living tissue. Especially, the synthetic hydrogels with controllable and reproducible properties were used as scaffolds to study the behaviors of cells in vitro and implanted test in vivo. In this review, two different structurally designed hydrogels, single-network (SN) hydrogels and double-network (DN) hydrogels, were used as scaffolds. The behavior of two cell types, anchorage-dependent cells and anchorage-independent cells, and the differentiation behaviors of embryoid bodies (EBs) were investigated on these hydrogels. Furthermore, the behavior of chondrocytes on DN hydrogels in vitro and the spontaneous cartilage regeneration induced by DN hydrogels in vivo was examined.

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I. INTRODUCTION

Hydrogels are three-dimensional cross-linked networks of water-soluble polymers that can be made from virtually any water-soluble polymer, thus encompassing a wide range of chemical compositions and bulk physical properties (Osada, 2000; Osada & Kajiwara, 2001). Since their three-dimensional network structures and viscoelasticities are similar to those of the macromolecular-based extracellular matrix (ECM) in biological tissue, hydrogels are used not only as artificial ECMs for repairing and regenerating a wide variety of tissues and organs but also as substitute material to develop artificial tissues and organs (Drury & Mooney, 2003; Degoricija et al. 2008; Berski et al. 2008; Weinberg & Bell, 1986; Chupa, Foster, Sumner, Madihally, & Mattew, 2000; Pieper, Hafmans, Veerkamp, & Van Kuppevelt, 2000; Park, Joung, Lee, Lee, & Park, 2008).

Some naturally derived hydrogels, such as agarose, chitosan, collagen, and hyaluronic acid (HA), can be used as tissue engineering scaffolds (Stokols et al. 2006; Li, Ramay, Hauch, Xiao, & Zhang, 2005; Madihally & Matthew, 1999; Sachlos, Reis, Ainsley, Derby, & Czemuszka, 2003). However, these hydrogels usually show poor cellular compatibility unless modified to include cell adhesive proteins. Our studies have shown that synthetic hydrogels with negative charges, such as poly (sodium p-styrene sulfonate) (PNaSS), or poly (2-acrylamido-2-methylpropanesulfonic sodium) (PNaAMPS), are effective scaffolds for culturing cells without the need for any surface modifications (Chen et al. 2005; Chen, Shen, Gong, & Osada, 2007; Chen et al. 2009; Chen, Ogawa, Kakugo, Osada, & Gong, 2009; Chen, Yang, & Gong, 2009). As scaffolds, hydrogels from synthetic polymers hold many advantages over natural polymers. Benefits of synthetic scaffolds include that they are infection-free, their low cost, can withstand high-temperature sterilization, have controllable and reproducible properties, and can mimic several properties of natural ECM to regulate bio-specific cell adhesion and cell migration.

In vivo, cells reside in a complex environment that has unique biochemical and biomechanical properties. Effective hydrogel scaffolds should therefore provide a similar environment for target cells. In our studies, we found that cell behavior on hydrogel scaffolds was strongly dependent on cell-cell and cell-hydrogel interactions, which could be regulated by hydrogel charge properties. In this review, the effects of hydrogel scaffolds on cell behavior are introduced. Two kinds of hydrogels, single-network (SN) hydrogels and double-network (DN) hydrogels, were used as scaffolds *in vitro* and *in vivo*. The behavior of two cell types, anchorage-dependent cells (endothelial cells) and anchorage-independent cells (chondrocytes), and the differentiation behaviors of embryoid bodies (EBs), were evaluated. Finally, results on DN hydrogels used for cartilage regeneration *in vitro* and *in vivo* are summarized.

II. HYDROGELS AS SCAFFOLD FOR TISSUE ENGINEERING: CELL BEHAVIOR IN VITRO

1. Single-Network Hydrogels

Neutral hydrogels, including polyacrylamide (PAAm) and poly *N, N*-dimethylacrylamide (PDMAAm), have no ionized group; negatively charged hydrogels, including PNaAMPS and PNaSS, have polymers with sulfonate group side chains, were used in these review. The chemical structures of these SN hydrogels are shown in Scheme 1 and were synthesized by radical polymerization as previously described (Chen et al. 2005; Chen et al. 2007; Chen et al. 2009; Chen et al. 2009; Chen et al. 2009) .

Human coronary artery endothelial cells (HCAECs), a type of anchorage-dependent cell,