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A dual-functional fibrous scaffold enhances P450 activity of cultured primary rat hepatocytes

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Abstract

We have designed a novel dual-functional electrospun fibrous scaffold comprising two fiber mesh layers that were modified differently to induce two separate biological responses from hepatocytes. The first fiber layer was galactosylated on the surface to mediate hepatocyte attachment, while the second layer was loaded with 3-methylcholanthrene (3-Mc) to enhance cytochrome P450 activity of hepatocytes. Primary rat hepatocytes cultured on the galactosylated fibrous scaffolds loaded with different concentrations of 3-Mc were compared for their cell attachment efficiency, albumin secretion activity and cytochrome P450-dependent 7-ethoxycoumarin *O*-deethylase activity. This hybrid fibrous scaffold mediated hepatocyte attachment with slightly lower efficiency ($76 \pm 2.3\%$) than a single-layer galactosylated fibrous scaffold ($84 \pm 3.5\%$). More importantly, the cytochrome P450 activity of the hepatocytes cultured on the hybrid scaffold correlated well with the 3-Mc loading level. The results also showed that transfer of 3-Mc to hepatocytes through direct cell–fiber contact was the dominant transport route, with the induced cytochrome P450 activity being 1.9- to 4.8-fold higher than that of transfer of 3-Mc to hepatocytes via dissolution from fibers to medium. This study demonstrates the feasibility of creating multi-functional fibrous scaffolds that serve both as an adhesive substrate and as a delivery vehicle for bioactive molecules.

Keywords: Electrospun fiber; Surface modification; Drug encapsulation; Hepatocyte culture

1. Introduction

Electrospun polymeric fibers have demonstrated their potential in many biomedical applications, including the production of scaffolds for tissue engineering and drug delivery. In the tissue engineering context, the topographical features provided by electrospun fibers play a significant role in regulating cell responses [1–4]. For example, mesenchymal stem cells cultured on electrospun fibrous scaffolds facilitated their differentiation into adipogenic, chondrogenic or osteogenic lineages, with corresponding increases in the expression of lineage-specific genes [1,2]. On axially aligned electrospun fibrous scaffolds, neuronal cells and cardiomyocytes have also been shown to grow axially along the fiber's orientation [3,4].

In order to improve cell–fiber interaction and the delivery of biochemical cues locally to the cells cultured on the fibrous scaffold, electrospun fibers can be further functionalized by surface conjugation or adsorption of bioactive molecules and/or by the loading of bioactive molecules in electrospun fibers. The surface functionalization method produces a fibrous scaffold that can interact with specific

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ligand receptors on cell membranes to induce specific cellular responses [5–7]. For example, Kim and Park demonstrated that cell attachment, spreading and proliferation of 3T3 fibroblasts were greatly enhanced in RGD peptide-immobilized electrospun fibrous scaffolds [6]. We have also demonstrated that electrospun fiber scaffolds with specific surface modifications enhance adhesion and phenotype maintenance of primary rat hepatocytes and hematopoietic stem/progenitor cells [5,7]. In contrast, the loading method provides fibers that can continuously release effector molecules to influence cell behavior [8,9]. For example, Liang et al. showed that controlled release of DNA nanoparticles released from doped electrospun fibrous scaffolds are effective in transfecting 3T3 fibroblasts in vitro [9]. We have also demonstrated previously that sustained release of heparin from electrospun fibrous scaffolds prevents the proliferation of vascular smooth muscle cells in culture [8].

In nature, cell survival, proliferation, differentiation and functions are regulated by a set of complex, spatially and temporally controlled milieu of biochemical and topographical cues emanating from the extracellular microenvironment; therefore, an electrospun fibrous scaffold with both surface functionality and incorporated biochemicals will be highly desirable in providing multiple types of bioactive cues to cultured cells. Functional maintenance of primary hepatocytes is critical in developing a bioartificial liver assist device, engineered liver tissue or a cell culture model for drug testing, though it is still difficult to achieve in vitro [10,11]. In particular, cytochrome P450 enzymatic activity, as a marker for biotransformation function, is rapidly lost in hepatocytes even on substrates that elicit good cell anchorage (e.g. Matrigel coating/sandwich configurations) or in hepatocyte aggregates (spheroids) [12–14].

3-Methylcholanthrene (3-Mc) has been shown to selectively induce a transient increase in P450 activity as it is a potent inducer for CYP1-dependent xenobiotic oxidation activities [14–17]. Typically, 3-Mc is repeatedly added in cell culture medium due to the poor solubility of 3-Mc in aqueous medium [18,19]. Bresnick et al. also showed that accumulation of 3-Mc in the liver tissue following intraperitoneal administration resulted in prolonged P450 activity [20]. The lipophilic nature of 3-Mc and its low solubility in medium make it more efficient at delivering 3-Mc locally. In this study, we investigate the effect of 3-Mc-encapsulated fibrous scaffold on P450 activity of culture hepatocytes.

In order to provide efficient delivery of 3-Mc to hepatocytes locally, it is preferable to adhere hepatocytes to a fibrous substrate. We have previously synthesized a galactosylated fibrous scaffold that mediates effective adhesion to primary hepatocytes [5]. Here we used a two-layered scaffold design, with each layer serving a different function (Fig. 1): one fiber layer is galactosylated, to mediate hepatocyte attachment, while the other layer is loaded with 3-Mc, to induce hepatocyte cytochrome P450 activity. The functions of rat hepatocytes cultured on hybrid fibrous scaffolds loaded with different amounts of 3-Mc are com-

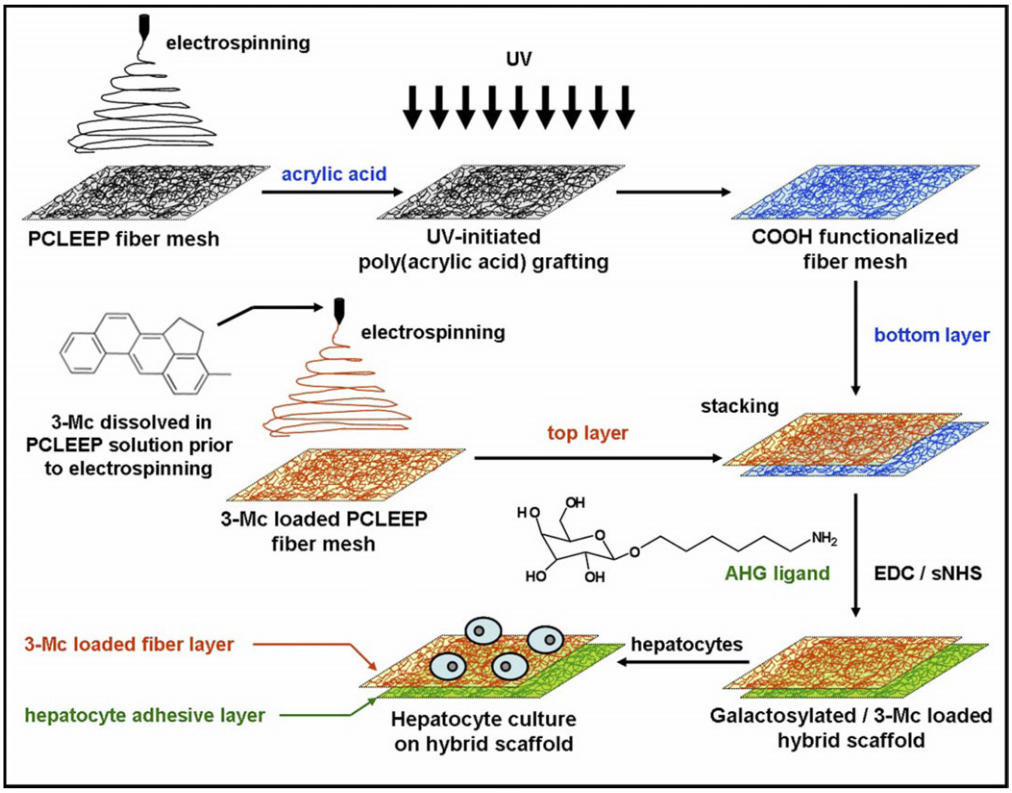

Fig. 1. Preparation of galactosylated/3-Mc-loaded electrospun PCLEEP fibrous scaffold.