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Chondrocyte behavior on nanostructured micropillar polypropylene and polystyrene surfaces

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ABSTRACT

This study was aimed to investigate whether patterned polypropylene (PP) or polystyrene (PS) could enhance the chondrocytes' extracellular matrix (ECM) production and phenotype maintenance. Bovine primary chondrocytes were cultured on smooth PP and PS, as well as on nanostructured micropillar PP (patterned PP) and PS (patterned PS) for 2 weeks. Subsequently, the samples were collected for fluorescein diacetate-based cell viability tests, for immunocytochemical assays of types I and II collagen, actin and vinculin, for scanning electronic microscopic analysis of cell morphology and distribution, and for gene expression assays of Sox9, aggrecan, procollagen α_1(II), procollagen α_1(X), and procollagen α_2(I) using quantitative RT-PCR assays. After two weeks of culture, the bovine primary chondrocytes had attached on both patterned PP and PS, while practically no adhesion was observed on smooth PP. However, the best adhesion of the cells was on smooth PS. The cells, which attached on patterned PP and PS surfaces synthesized types I and II collagen. The chondrocytes' morphology was extended, and an abundant ECM network formed around the attached chondrocytes on both patterned PP and PS. Upon passaging, no significant differences on the chondrocyte-specific gene expression were observed, although the highest expression level of aggrecan was observed on the patterned PS in passage 1 chondrocytes, and the expression level of procollagen α_1(II) appeared to decrease in passaged chondrocytes. However, the expressions of procollagen α_2(I) were increased in all passaged cell cultures. In conclusion, the bovine primary chondrocytes could be grown on patterned PS and PP surfaces, and they produced extracellular matrix network around the adhered cells. However, neither the patterned PS nor PP could prevent the dedifferentiation of chondrocytes.

1. Introduction

Cell culture surface materials have been shown to have a significant role in interactions controlling the cellular behavior. For instance, micro- and nanoscale surface topographies influence cell behavior [1,2]. Surface qualities are known to affect the cellular properties, such as adhesion [3–5], morphology [6,7], orientation [8,9] and proliferation [4,10]. For instance, it has been shown that the nanocrystalline diamond with grain size less than 100 nm enhanced human osteoblast adhesion, while the microcrystalline diamond inhibited it [4]. Similarly, the proliferation of the human osteoblasts was greater on nanocrystalline than on macrocrystalline diamond [4]. The organization of cytoskeleton and cell morphology of the human fibroblasts also varied between nano-island and planar surfaces within the cell culture time [11].

Chondrocyte, a specialized cell in articular cartilage, is obligatory for cartilage formation and functionality. Articular cartilage has a low repair capacity and, therefore, it is progressively worn out in injured and osteoarthritic joints. Cell-based therapies have been considered for the treatment of cartilage lesions, and autologous chondrocyte implantation (ACI) has been used clinically to repair cartilage defects worldwide [12]. However, the chondrocyte source for ACI is always problematic because of the low cell density in the articulr cartilage, invasive manner of cell isolation and dedifferentiation of chondrocytes in expansion culture [13–15]. During this process of de-differentiation, the cells change their phenotype in monolayer culture by adopting fibroblast-looking morphology, decreasing their ability to produce proteoglycans, and switching the expression of type II collagen to type I collagen during passaging [14,15].

The important role of cell shape in the modulation of the chondrocyte phenotype was originally demonstrated in suspension cultures on agarose [13]. High-extension silicone rubber dishes also inhibited the chondrocyte dedifferentiation of continuously expanded bovine primary chondrocytes [16]. The present knowledge on tissue engineering suggests that preservation of chondrocytic phenotype in vitro requires three-dimensional culture models, which in fact support the round

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shape of chondrocytes. It can be speculated that the adhesion, phenotype and cellular shape of chondrocytes are related to each other.

Previously, it was shown that the human primary osteogenic sarcoma cells (Saos-2) cultured on nanostructured polypropylene micropillars (patterned PP) tended to grow in cell clusters, showing an apparent three-dimensional structure [17]. In this study, we surmised that a nanostructured micropillar of PP (patterned PP) might enhance the chondrocyte extracellular matrix (ECM) production and retain the chondrocytes' phenotype when the bovine primary chondrocytes are cultured on top of the patterned PP. Similarly patterned polystyrene (PS) was also used in the study to compare their effects on cellular adhesion and ECM production properties, as well as the capacity of different material types to maintain the chondrocytic phenotype. Smooth PP and PS surfaces were tested, too.

2. Materials and methods

2.1. Materials

Aluminum foils (Al, 4.2 × 3.3 cm, 0.25 mm thick, Alfa Aesar, Puratronic, 99.997%) were degreased by ultrasonication in acetone, electropolished in perchloric acid:ethanol (1:8 v/v, platinum as counter electrode), and then used as starting templates for further structuring and injection molding. Polypropylene homopolymer (HD 120 MO, 908 kg/m^3) and polystyrene (Empera® 116 L) were purchased from Borealis Polymer Ltd. (Porvoo, Finland), and were used directly in the injection molding process.

2.2. Preparation of the micro- or nano-structures

The micro- or nano-structures on aluminum foils were obtained by using RP-1AH micro-working robot (Mitsubishi Electric, Japan) with tungsten carbide needles (Gritech Ltd Joensuu, Finland). The micro-working robot has a CR1 control unit, and a feedback unit, which was provided by Delta Enterprise Ltd (Espoo, Finland). Scanning electron microscopy (SEM) images of the aluminum foils were taken with a Hitachi S4800 field emission SEM microscope (Japan). Samples were sputter coated with a 2 nm gold layer (Cressington 208HR high resolution sputter coater, Walford, England), and taped onto a stub.

The contact angles (θ) on polymer discs were measured with a KSV Cam 200 (Helsinki, Finland) contact angle meter with both deionized water and cell culture medium solution at room temperature. The values were determined by fitting the droplet profiles to Young–Laplace equation.

2.3. Fabrication of the polymer surface structures

The fabrication method of the polymer surface structures follows a previous study [18]. The aluminum foil was structured first by a micro-working robot to create micro-sized structures. It was then anodized for 24 h (0.3 M oxalic acid, 3 °C) to obtain nano-scaled structures. After that, the structured foil was glued onto a steel plate and used as mold insert. The polymer surface structure was obtained by using the mold insert in injection molding process. The working procedures and dimensions of the injection molded PP or PS discs are shown in Fig. 1.

2.4. Sterilization of the micro- or nano-structured disc

The smooth and patterned PP and PS discs were first sterilized for approximately 2 h in 75% ethanol, then washed with sterile PBS, and subsequently with chondrocyte culture medium described below. The discs were left to dry for a while. Finally, the discs were placed in 6-well plate under the sterilized Teflon ring to make them ready for cell seeding.

2.5. Isolation and cultivation of bovine primary chondrocytes

Primary chondrocytes were isolated with collagenase digestion from femoral condyles of 18–22-month-old healthy cows as described previously [19]. Bovine joints were provided by the local abattoir (Atria, Kuopio, Finland). After isolation, the chondrocytes were seeded on the sterilized smooth PP and PS discs, as well as on patterned PP and PS discs suspended in chondrocyte culture medium [Dulbecco's Modifies Eagle's Medium high glucose (4.5 g/l glucose, Sigma-Aldrich, Ayrshire, UK) supplemented with 10% fetal bovine serum, penicillin (100 U/ml, Euroclone, Pero, Italy), streptomycin (100 μg/ml, Euroclone), 2 mM L-glutamine (PAA, Pasching, Austria), and 50 μg/ml L-ascorbic acid 2-phosphate trisodium salt (Fluka, Buchs, Switzerland)] at the cell density of 0.2 × 10^6 for cell viability assay, immunocytochemical assay, scanning electron microscopy and quantitative real time RT-PCR analysis. The cells were cultured in the chondrocyte culture medium for 2 weeks before the analyses. All the experiments were repeated at least 3 times.

2.6. Cell viability assay

The cell viability of the chondrocytes cultured on the patterned PP and PS was examined by using a fluorescent probe. After a 2-week cultivation period, the cells were incubated for 5 min with a solution containing 10 μM of fluorescein diacetate (Sigma-Aldrich, Ayrshire, UK) and 60 μM propidium iodide (Sigma-Aldrich) dissolved in PBS. After washing with PBS, the cells were observed with a fluorescence microscope (IX-70, Olympus, Tokyo, Japan), and photographed. The cell proliferation on various surfaces was assayed with MTT colorimetric method, measuring the metabolic activity of mitochondria as described previously [7].

2.7. Scanning electron microscopic analysis

Scanning electron microscopic imaging was performed with XL30 ESEM TMP (PEI Company Philips Ab, Eindhoven, the Netherlands) as described previously [7]. After a 2-week cultivation, the adhered cells on the smooth and patterned PP and PS were fixed with 2.5% w/v glutaraldehyde (Sigma) in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at

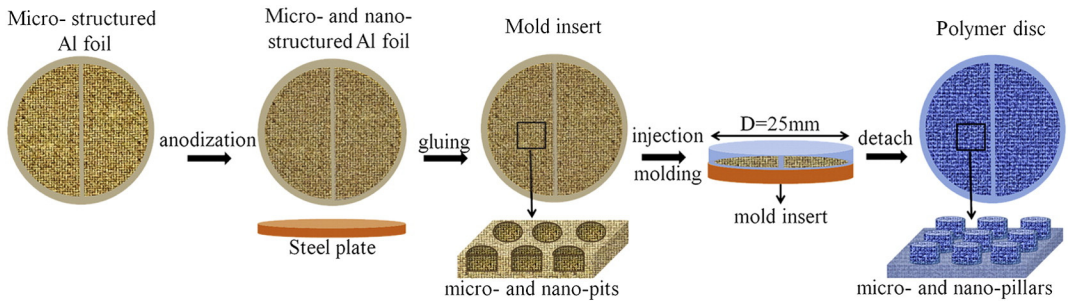

Fig. 1. Fabrication procedures of polymer surface structures.