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Assessment of stem cell/biomaterial combinations for stem cell-based tissue engineering

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Abstract

Biomaterials are used in tissue engineering with the aim to repair or reconstruct tissues and organs. Frequently, the identification and development of biomaterials is an iterative process with biomaterials being designed and then individually tested for their properties in combination with one specific cell type. However, recent efforts have been devoted to systematic, combinatorial and parallel approaches to identify biomaterials, suitable for specific applications. Embryonic and adult stem cells represent an ideal cell source for tissue engineering. Since stem cells can be readily isolated, expanded and transplanted, their application in cell-based therapies has become a major focus of research. Biomaterials can potentially influence e.g. stem cell proliferation and differentiation in both, positive or negative ways and biomaterial characteristics have been applied to repel or attract stem cells in a niche-like microenvironment. Our consortium has now established a grid-based platform to investigate stem cell/biomaterial interactions. So far, we have assessed 140 combinations of seven different stem cell types and 19 different polymers performing systematic screening assays to analyse parameters such as morphology, vitality, cytotoxicity, apoptosis, and proliferation. We thus can suggest and advise for and against special combinations for stem cell-based tissue engineering.

Keywords: Mesenchymal stem cells; Preadipocytes; Endothelial progenitor cells; Dental pulp stem cells; Hematopoietic stem cells; Embryonic stem cells

1. Introduction

In modern medicine, natural and synthetic biomaterials play an increasingly important role in the treatment of diseases and the improvement of health care [1]. To date, many biomaterials, such as titanium, polyetherurethane or polydimethylsiloxane are used routinely. The development of novel "smart" biomaterials with optimized characteristics for very specific applications has become a main research focus [1–4]. For tissue-engineering applications, biomaterials often serve as scaffold for a specific cell type. An ideal scaffold should provide chemical stability or degradability and physical properties matching the surrounding tissue to

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provide cytocompatibility, support adhesion, proliferation, stability, and mechanical strength. The adaptation of biomaterials for tissue-engineering applications is an iterative process: Usually a biomaterial is tested in combination with only one specific cell type. More recently, combinatorial approaches have been employed to identify biomaterials suitable for specific applications. In a landmark study, Langer et al. [5] produced a biomaterial array consisting of 576 individual polyacrylate spots. This array allowed for the simultaneous analysis of hundreds of cell–polymer combinations on a single microscopic slide. However, the robotic synthesis strategy limited the choice of biomaterials to relatively innoxious reacting solvents around ambient temperature. The parallel analysis of thermoplastic polymers, metals or ceramics that make up the majority of biomaterials currently in medical use was precluded. Further studies described the use of biomaterial arrays consisting of polymers or extracellular-matrix molecules [6,7]. Such arrays can be used for a high throughput screening of cell–biomaterial interactions and thus to identify materials supporting a specific cell function. Traditionally, cell–material studies are limited to few materials and an established cell line or a single-cell type. A relatively novel tissue-engineering concept advocates the use of scaffolds specifically designed to differentiate precursor cells or even stem cells into a defined phenotype *in situ* at the implantation site. To identify scaffolds with such innovative properties requires the testing of a maximum number of cell–material combinations with subsequent unbiased evaluation of cell proliferation and differentiation.

Nowadays, stem cells represent a particularly attractive cell type for tissue-engineering applications. Stem cells are characterized by two unique properties in one cell: their high self-renewal activity and their multilineage differentiation potential, which make them an ideal source for cellular therapy and regenerative medicine. These cells can be expanded *in vitro* and differentiated into diverse cell types, processes that can be supported or induced by biomaterials [8].

Parameters such as surface topography, chemistry (physicochemical property) including surface wettability (surface energy) and surface charge strongly influence cell–material interactions [9]. So far, no general principles are known that allow a prediction of the extent of cellular behaviour on a given biomaterial [10]. Therefore, cell adhesion, morphology, vitality, proliferation, cytotoxicity, and apoptosis have to be analysed and matched into a basic assessment.

We here introduce a grid-based platform for the assessment of stem cell–biomaterial interactions. We chose several stem cell types and thus compared pluripotent embryonic vs. multipotent adult stem cells (mesenchymal stem cells, preadipocytes, dental pulp stem cells, hematopoietic stem cells, and endothelial progenitor cells). All adult stem cell types are of mesodermal origin, but are precursors for different specialized cell types. We established a biomaterial bank, comprising established and newly developed polymers, but also allowing testing of ceramics and ceramic polymer blends.

We report the systematic screening of 140 different combinations of stem cells and polymers and demonstrate the usefulness of multifactorial analyses in the testing of cell–material combinations.

2. Materials and methods

2.1. Materials

2.1.1. Biopolymers, degradable

2.1.1.1. Alginate. Alginic acid sodium salt (Algin, sodium alginate) from brown algae (*Macrocystis pyrifera*) was purchased in *BioChemika* quality from Sigma-Aldrich Chemie GmbH (Germany). Alginate films were produced by adding 20 ml of an aqueous 1% (w/v) sodium alginate solution to 0.5 ml of 0.01 M $CaCl_2$. Films were then stabilized by crosslinking alginate molecules with six-arm star-shaped isocyanate-terminated poly(ethylene glycol) (IPDI-starPEG, Mw: 18,000 g/mol, SusTech GmbH & Co. KG Darmstadt, Germany) [27]. Alginate films were disinfected by spraying with 70% ethanol, washed six times with aqua bidest and finally six times with phosphate buffered saline (PBS, pH 7.4) before being placed in cell culture dishes of tissue culture polystyrene (TCPS, Greiner bio-one, Germany). Unless otherwise noted, this disinfection procedure was used for all other materials.

2.1.1.2. Collagen. The constant quality collagen matrices used in this study are large scale commercial products by Dr. Suwelack Skin & Health Care AG (Billerbeck, Germany). A collagen suspension containing collagen types I, III, and V was isolated from bovine skin (bovine spongiform encephalopathy tested, BSE tested). The dermal collagen was extensively highly purified and freeze-dried resulting in a sponge-like matrix structure. Collagen samples were taken from collagen sheets (1 mm thick) using a hole punch. For cell culture experiments, porous collagen scaffolds were transferred into non-porous sheets by compression between two stainless steel plungers and 10 tonne pressure at ambient temperature.

2.1.1.3. Fibrin. One hundred and eighty microlitre of a sterile fibrinogen-suspension consisting of 50 µl $CaCl_2$ (50 mM, Roche, Mannheim, Germany), 120 µl GBSH5 buffer (without glucose and Ca^{2+}), and 830 µl fibrinogen (20 mg/ml, Sigma, Steinheim, Germany) was mixed with 20 µl thrombin (10 units/ml, Sigma, Steinheim, Germany) and poured in cell culture plates to polymerize [15].

2.1.1.4. Hyaluronic acid. Potassium hyaluronan (hyaluronic acid potassium salt, potassium hyaluronate, HA) from human umbilical cord with a protein content less than 2% (w/v) was purchased by Sigma-Aldrich Chemie GmbH (Germany). HA films were produced by mixing a 0.7% (w/v) solution of potassium hyaluronan (Mw: 750,000 g/mol) with a 15% (w/v) solution of IPDI-starPEG (Mw: 18,000 g/mol, SusTech GmbH & Co. KG Darmstadt, Germany) [27]. Freshly prepared solutions were mixed and directly casted onto a glass plate. Foil thickness was adjusted by using a scraper to approximately 600 µm.

2.1.2. Degradable synthetic polymers

2.1.2.1. BAK 1095. Polyesteramide type BAK 1095 was a gift from Bayer MaterialsScience AG (Germany). The degradable polymer was synthesized in an industrial plant, extruded and transferred into granules successively. The polymer was prepared by a two-step (ring-opening polymerization (ROP) and polycondensation) one-batch reaction [28]. Foil production was established by a melt-press process. Polymer granules were ground to powder in a cryo-mill. 1.2 g polymer powder per foil was placed between Teflon-covered metal plates, temperature was raised to 180 °C and maintained for 5 min. A load of 1 tonne was applied for 9 min. After cooling to room temperature the prepared foil was taken out of the