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Bioreactor design for cornea tissue engineering: Material–cell interactions

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Abstract

Several materials were evaluated for potential use in a bioreactor system for a tissue-engineered cornea. Two types of cytotoxicity tests were performed using human corneal stromal fibroblasts: a 24 h cytotoxicity test based on the ASTM standard F813-01 and a 7 days growth inhibition test. It was determined that culture configuration, autoclaving and materials surface preparation were all important factors influencing cell viability. Poly(etheretherketone) and titanium–6Al–4V were found to be the most appropriate materials for use in a corneal bioreactor system. Furthermore, poly(oxymethylene) copolymer and poly(tetrafluoroethlylene) are not safe for use with human corneal fibroblasts after autoclaving.
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1. Introduction

Diseases and injuries to the cornea are common around the world and while corneal blindness may sometimes be repaired via corneal transplants, there is a worldwide shortage of viable donor tissue [1]. The focus of our work is on the development of a tissue-engineered (TE) cornea which has the potential to reduce the demand for donor corneas and result in lower rejection rates following the transplant [2]. A major hurdle in creating a TE corneal stromal equivalent is controlling the wound healing differentiation response of corneal stromal fibroblasts in vitro. In a healthy cornea, the stromal cells primarily express a quiescent keratocyte phenotype which is partially responsible for maintaining the transparency of the tissue [3]. When seeded on a three-dimensional collagen scaffold, however, these cells primarily take on the repair fibroblast or myofibroblast phenotype [4]. Myofibroblasts and repair fibroblasts do not express the corneal crystallin proteins transketolase (TKT) and aldehyde dehydrogenase 3 (ALDH3), which have been shown to reduce light scattering by the cell [5]. One potential method for controlling the phenotype of these cells in vitro is through the application of mechanical stress to the cells. Studies have shown that placing cells in an environment that mimics the in vivo stress conditions can result in more functional tissue equivalents [6]. The authors have designed a bioreactor system to impart simulated intraocular pressure (and therefore the corresponding hoop stress) on cells seeded in three-dimensional collagen scaffolds in order to improve the transparency of the engineered corneal tissue. An important aspect of the design and manufacturing of any biomedical device is materials selection. For our bioreactor to function properly, the material in contact with the cells must be biocompatible, sterilizable and machinable.

Research has been conducted by others on the relative biocompatibilities of various materials for use in human implant applications [7–10]. While this study is focused on selecting a material specifically for use in an in vitro

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bioreactor system, the biocompatibility and mechanical properties of implant materials indicate that a number of these materials may be candidates for our application. Polymers, e.g., poly(oxymethylene) homopolymer (POM-h – Delrin® acetal resin is a well-known commercial form) and ultra high molecular weight poly(ethylene) (UHMWPE) have been commonly used in implants with good results [11,12]. Stainless steel 316L (SS 316L) and titanium–6Al–4V (Ti–6–4), have also been successfully used as implant materials [12]. Poly(tetrafluoroethylene) (PTFE – one commercially well-known form is the Teflon® fluoropolymer) was originally used as a material for total hip replacements [13]. However, concerns over wear and toxicity led to the replacement of PTFE with UHMWPE in later designs [12]. Despite the negative results in prosthetic hips, several studies have found PTFE to have a high cytocompatibility and have even used Teflon® fluoropolymer as a positive control for in vitro cytocompatibility studies [14,15]. The polymer poly(etheretherketone) (PEEK) is being considered a strong candidate for spinal implants [16]. Despite the successful clinical applications of these materials, there have been indications that the effects of a material on cells in culture depend on the cell type under investigation [11]. Penick et al. [11] found that POM-h performed well in their bone-marrow-derived mesenchymal stem cell perfusion bioreactor, while LaIuppa et al. [17] reported that the same material was found to be cytotoxic when hematopoietic progenitor cells were grown directly on the surface.

All implantable materials must be sterilized prior to use. The easiest and most available sterilization method is steam sterilization, or autoclaving. Steam sterilization, as with all sterilization methods, has the potential to have detrimental effects on a material. Studies have shown that sterilization can change the chemical and physical properties of a material [7,18–20]. This can result in degradation and the release of potentially toxic monomers and plasticizers from the material [11]. Poly(ethylene terephthalate) (PET), for example, released a cyclic trimer and had a reduced molecular weight following steam autoclaving [18]. PET was also found to reduce cell viability after 30 min of steam sterilization [18]. Similar results were found for poly(vinyl chloride) (PVC) [18]. Sterilization can also cause changes in the actual chemical structure of a material, e.g., UHMWPE undergoes oxidation during steam sterilization [21]. Steam sterilization was found to reduce the biocompatibility of titanium and the effects became more drastic with repeated sterilization [19].

Cell culture techniques are commonly used to determine the cytotoxicity of materials prior to studying the in vivo effects of implant materials and medical devices [12,22,23]. Two different culture configurations are discussed within our work. One method of measuring the cytotoxicity of materials for use in medical devices via direct contact with cells in monolayer is described in the ASTM standard F 813-01 entitled "Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices" (hereafter referred to as the "cytotoxicity configuration"). Here, a cell layer is grown to near confluence before a candidate material is placed into the well. The potential cytotoxic effects are evaluated 24 h following the addition of the material to the cell culture by using light microscopy to assess cell viability. A second approach evaluates the potential growth inhibition caused by the sample (hereafter referred to as the "growth inhibition configuration"). In this test, cells are seeded in the presence of the sample and remain in culture together with the material for 7 days. This approach may allow for the isolation of the chemical effects of the material from the combined chemical and mechanical effects of the material on the cell culture. The results of both the cytotoxicity test and the growth inhibition test can be quantified using standard cell viability assay techniques.

In this study, the growth inhibition and cytoxicity configurations were used to test the cytocompatibility of the following selected materials after autoclaving: POM-h, poly(oxymethylene) copolymer (POM-c), PEEK, UHMWPE, PTFE, SS 316L and Ti–6–4. In order to ensure that the material selected is compatible for our application, human corneal stromal fibroblasts (HCF) were used to eliminate any effects of cell type dependence. This approach has not only allowed us to determine which materials are suitable for our application, but also to elucidate differences resulting from various material preparations, the 2 different culture configurations and potential autoclave dependencies.

2. Materials and methods

2.1. Cell culture

HCF were isolated from corneas obtained from the MN Lions Eye Bank according to published methods [24]. Cells were then cultured and passaged in T-150 cell culture flasks in DMEM/F12 media (Sigma, St. Louis, MO) containing 10% fetal bovine serum (FBS; Sigma, St. Louis, MO) and 1% penicillin/streptomycin (500 units penicillin and 0.5 mg streptomycin; Sigma, St. Louis, MO). All cells were grown in an incubator at 37 °C and 5% CO_2. All cells used in this study were at passage 8 or less.

Cells were harvested by adding 10 ml of 0.05% trypsin with EDTA (Invitrogen, Carlsbad, CA) to each confluent T-150 flask and incubating until the cells had dislodged from the culture surface. The trypsin was neutralized by adding 20–30 ml of DMEM/F12 media with 10% FBS. Cells were then centrifuged, re-suspended in 1 ml of culture media and counted using a hemocytometer. Cells were then seeded into six-well plates at a density determined by running an MTT calibration test plate for the growth inhibition configuration (see Section 2.4) or 3500 cells cm^{-2} for the cytotoxicity configuration.

As discussed in the Section 1, 2 different cell culture configurations were used in this study. Cells were grown in the presence of the candidate materials for 1 day (cytoxicity

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