Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 1211–1218
Biomaterials
www.elsevier.com/locate/biomaterials

Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation

Christopher G. Williams[a,b,1], Athar N. Malik[a,1], Tae Kyun Kim[a,c], Paul N. Manson[b], Jennifer H. Elisseeff[a,*]

[a] Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[b] Division of Plastic and Reconstructive Surgery, Department of Surgery, Johns Hopkins Hospital, Baltimore, MD 21287, USA
[c] Department of Orthopedic Surgery, Seoul National University Bundang Hospital, South Korea

Received 29 October 2003; accepted 18 April 2004

Available online 21 July 2004

Abstract

The development of biocompatible photopolymerizing polymers for biomedical and tissue engineering applications has the potential to reduce the invasiveness and cost of biomaterial implants designed to repair or augment tissues. However, more information is needed about the cellular toxicity of the compounds and initiators used in these systems. The current study evaluates the cellular toxicity of three ultraviolet sensitive photoinitiators on six different cell populations that are used for engineering numerous tissues. The photoinitiator 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959) caused minimal toxicity (cell death) over a broad range of mammalian cell types and species. It was also demonstrated that different cell types have variable responses to identical concentrations of the same photoinitiator. While inherent differences in the cell lines may contribute to the variable cytotoxicity, a correlation between cellular proliferation rate (population doubling time) and increased cytotoxicity of the photoinitiator was observed. Cell lines that divided more quickly were more sensitive to photoinitiator-induced cell death. In summary, the photoinitiator Irgacure 2959 is well tolerated by many cell types over a range of mammalian species. Cell photoencapsulation strategies may be optimized to improve cell survival by manipulating proliferation rate.

Keywords: Photopolymerization; Cytotoxicity; Hydrogel; Cell encapsulation; Cell proliferation; Free radical

1. Introduction

Photopolymerization is used in a broad range of commercial and biological applications such as printing, dentistry, optical materials [1], encapsulating pancreatic islet cells [2,3], and blood vessel adhesives [4]. These systems are being used for an increasing number of biomedical applications because of their ability to rapidly convert liquid monomer or macromer solutions to a cross-linked network under physiologic conditions [5]. Important advantages of photopolymerizable systems include powerful spatial and temporal control of reactions kinetics, minimal heat production, ability to uniformly encapsulate cells, and significant adaptability for in situ polymerization by adapting light sources to fit clinical scenarios.

The development of biocompatible photopolymerizable polymers for tissue engineering applications has the potential to reduce the invasiveness and cost of many surgical procedures. For instance, instead of making large incisions to implant cell- and polymer-based therapies, physicians could potentially reduce costs and surgical trauma by injecting and efficiently polymerizing cells and polymers in situ in tissue defects by minimally invasive procedures. If fully developed into clinical practice, such a strategy could postpone or eliminate the need for more aggressive corrective therapies.

Photopolymerization reactions are driven by chemicals that produce free radicals when exposed to specific wavelengths of light. A variety of photoinitiators, each

*Corresponding author.
E-mail address: jhe@bme.jhu.edu (J.H. Elisseeff).
[1] Christopher G. Williams and Athar N. Malik contributed equally to this work.

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.04.024

with its unique absorption spectrum, are available and more are continually being developed. A photon from a light source excites or dissociates the photoinitiator into a high-energy radical state. This radical then induces the polymerization of a macromer solution [5]. However, the creation of a high-energy radical species in this system creates the potential for oxidative damage to the cell populations that are photoencapsulated. Free radicals can cause damage to cell membranes, nucleic acids, and proteins, which can lead to cell death [6–8]. Furthermore, cancer researchers have demonstrated how oxidative damage can vary depending on cell type and proliferation rate [9], suggesting that photoinitiators may have varying effects on different cell types. Several groups, however, have made these systems cytocompatible and useful for in vitro and in vivo tissue engineering applications [5,10–16].

The goals of this paper were to define the toxicity profiles of three commonly used ultra violet light-sensitive photoinitiators on six different cells lines that have important potential in future tissue engineering applications. The photoinitiator with minimal toxicity was further characterized by investigating the relationship between photoinitiator sensitivity and cellular proliferation rate. The cytotoxicity profiles of 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Irgacure 2959), 1-hydroxycyclohexyl-1-phenyl ketone (Irgacure 184), and 2,2-dimethoxy-2-phenylacetophenone (Irgacure 651) on bovine chondrocytes, goat bone marrow-derived mesenchymal stem cells, human bone marrow-derived mesenchymal stem cells (Clonetics), rabbit corneal epithelial cells (ATCC), human fetal osteoblasts [17] (ATCC) and human embryonic germ cells (LVEC cell line) were evaluated [18].

2. Materials and methods

2.1. Cell culture

2.1.1. Human fetal osteoblasts (hFOB)

Cryopreserved human fetal osteoblasts [17] (hFOB 1.19, CRL-11372, ATCC, Manassas, VA) were purchased and stored in liquid nitrogen until needed per manufacturer's protocol. The cells were cultured in medium consisting of 1:1 mixture of DMEM and Ham's F-12 medium with 2.5 mM L-glutamine (ATCC) supplemented with 10% FBS (ATCC), 0.3 mg/ml Geneticin (Invitrogen, Carlsbad, CA).

2.1.2. Bovine chondrocytes (BC)

BC were isolated from full thickness explants from the femoral-patellar groove and femoral condyles of young calves (Research 87, Marlboro, MA) as described elsewhere [19]. Cells were stored in liquid nitrogen until needed. They were allowed to thaw and recover for 2–3 days in monolayer culture using chondrocyte medium (Dulbecco's Modified Eagle Medium (Gibco, Invitrogen), supplemented with 10 mM HEPES (Gibco, Invitrogen), 0.04 mM L-Proline (Sigma, St. Louis, MO), 50 mg/l ascorbic acid 2-phosphate (Sigma), 10% FBS (Hyclone, Utah), 1X penicillin–streptomycin (Gibco, Invitrogen)) prior to being used for experiments. Freezing medium consisted of the above described chondrocyte medium supplemented with 10% DMSO.

2.1.3. Corneal epithelial cells (statens seruminstitut rabbit cornea (SIRC), Ocyctolagus cuniculus)

Rabbit corneal epithelial cells, "SIRC cells," (CCL-60, ATCC) were purchased and stored in liquid nitrogen until needed per manufacturer's protocol. Cell culture medium consisted of minimum essential medium (Eagle) with Earle's balanced salt solution (ATCC) supplemented with 10% calf serum (ATCC).

2.1.4. Human mesenchymal stem cells (hMSC)

Human bone marrow-derived mesenchymal stem cells (hMSCs) (BioWhittaker, Cambrex, Walkersville, MD) were purchased and stored in liquid nitrogen until needed per manufacturer's protocol. Cells were cultured in mesenchymal stem cell growth medium (MSCGM; BioWhittaker Inc., Walkersville, MD).

2.1.5. Goat mesenchymal stem cells (gMSC)

Bone marrow from the femurs of three to three-and-a-half year old, castrated male goats being sacrificed for other reasons was aspirated into 10 ml syringes with 6000 units of heparin and processed within 4 h of harvest. The marrow samples were washed and centrifuged twice at 50*g* for 10 min in MSCGM prior to suspension in fresh MSCGM. The number of mononuclear cells was counted with a hemocytometer and plated in 75 cm^2 tissue culture plastic flasks at a density of approximately 120,000 mononuclear cells/cm^2. MSCs were frozen in liquid nitrogen in 50% MSCGM, 40% FBS (Hyclone, Logan, UT), 10% DMSO (Sigma, St. Louis, MO) until needed. Culture medium consisted of MSCGM.

2.1.6. Human embryonic germ cells (LVEC cell line)

The LVEC cell line was obtained as a gift from Shamblott et al. [18,20] and kept frozen in liquid nitrogen until use. Cells were expanded on collagen-coated tissue culture plastic 100-mm dishes in EGM-2-MV medium (BioWhittaker, Cambrex) and trypsinized when needed.

Frozen cells were thawed, plated in 75 or 175 cm^2 flasks (Falcon, Becton Dickinson, Franklin Lakes, NJ) in the appropriate medium, and grown at 37°C until 80–90% confluent. Cell viability after thawing was consistently above 90%. The BC were allowed to recover from cryopreservation for 2–3 days but were harvested