Chapter 6

Polymer Based Biointerfaces: A Case Study on Devices for Theranostics and Tissue Engineering

Pamela Mozetic, Mariarosaria Tortora, Barbara Cerroni, and Gaio Paradossi

6.1 Introduction

In the design of new devices supporting biomedical applications, the focus on the processes occurring at the interface with cells is a major issue. In this context, our interest on the formulation of new biomaterials and on the synthesis of next-generation ultrasound contrast agents (UCAs) lead us to the in vitro study to assess the biocompatibility of these novel devices. As UCAs are designed for parenteral administration, the time response of the primary immune system, mainly macrophages, should be addressed.

Our UCA [2], a poly (vinyl alcohol), PVA, based microbubble, has been designed as an injectable multifunctional device suitable for diagnostics as well as therapeutic treatment promoted by insonification. PVA is a polymer already used for biomedical applications. However, in contrast to other PVA based biomaterials, we have crosslinked modified PVA chains [1] carrying out a one-pot reaction in aqueous medium without introducing external crosslinkers that could in principle jeopardize the biocompatibility of the starting PVA material (see also chapter “Design of novel polymer shelled ultrasound contrast agents: toward a ultrasound triggered drug delivery.”). This reaction can be carried out without stirring the reaction medium or foaming the medium by applying a high shear rate stirring. In the first case a thin hydrogel membrane is obtained whereas an aqueous dispersion of microbubbles, MBs, is the final product of the latter case. The polymer shell of the microbubbles is expected to have the same chemical features as the membrane hydrogels. For this reason we have addressed our attention to biocompatibility studies of both hydrogels and microbubble systems as biointerface assessment is key in any application involving biomedical applications. We addressed in vitro biocompatibility study toward fibroblasts and macrophages as cell types having direct interaction with polymer surfaces in the blood stream.

When studying the processes occurring between microbubbles and cells, the establishment of the biointerface is a major issue as the floating tendency of microbubbles does not allow effective contact with cells. In order to
accomplish such contact, all polymeric microballoons were transformed into microcapsules by equilibrating them in ethanol followed by reconditioning in PBS buffer. In this way the air-filled microballoons were converted into sterile microcapsules having the same dimensions as the starting MBs [3]. Due to the increased density of the new particles with a core containing PBS, the microcapsules can be brought into contact with cells easily. This choice was dictated by the need to accomplish close contact of the cells with the sterile polymer devices, assuming that the chemical and physical surface features of the microcapsules are maintained during the microballoon-to-microcapsule conversion.

6.2 Materials and methods

6.2.1 Reagents

Dulbecco’s modified eagle medium (DMEM), L-glutamine 200 mM and penicillin/streptomycin solution (10000 U/ml and 10 mg/ml, respectively) were obtained from HyClone. Fetal bovine serum, Giemsa staining, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and phosphate buffered saline (PBS) and HBSS were purchased from Sigma Aldrich. Live/Dead viability/cytotoxicity kit was obtained from Molecular Probes, Invitrogen.

6.2.2 PVA hydrogels and MBs biocompatibility on NIH3T3 fibroblast mouse cell line

MBs fabricated at pH5 and room temperature (MBpH5RT), at pH5 and at 4°C (MBpH5C) and at pH2 and at 4°C (MBpH2C) were used throughout this study. MBs were functionalized with Arg-Gly-Asp, RGD, tripeptide as described in the chapter “Design of novel polymer shelled ultrasound contrast agents: toward a ultrasound triggered drug delivery”. All types of MBs were sterilized by EtOH 70% and re-suspended in sterile PBS buffer.

6.2.3 NIH3T3 fibroblasts and RAW 264.7 macrophage cultures

NIH3T3 mouse fibroblasts from Istituto Sperimentale Zoprofiattico della Lombardia e dell’Emilia Romagna (Italy), were maintained in Dulbecco’s modified Eagle medium (DMEM) containing 10% FBS, 1% penicillin/streptomycin solution and 1% glutamine at 37°C in a 5% CO₂ atmosphere. Cell viability and cell proliferation were determined by MTT assay [4].

RAW 264.7 macrophages were purchased from the Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna (Italy). The cells were maintained in complete medium consisting of DMEM supplemented with 2 mM L-glutamine, 10% heat-inactivated fetal bovine serum and 1% antibiotic mixture, consisting of 100 U/ml of penicillin and 100 μg/ml of streptomycin.