BIOCOMPATIBILITY SCREENING OF BIOMATERIALS FOR BONE TISSUE ENGINEERING – STUDY OF THE OSTEOGENIC CELL MORPHOLOGY AND ATTACHMENT BEHAVIOUR IN VITRO

Shareen Aini S¹, Mohamed Nainar S², Shahida Begum³, Ansari MNM³, Vicki WV², Hoque ME³, Ng MH¹* and Ruszymah BHI¹

¹Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur
²Centre for Advanced Materials, Universiti Tenaga Nasional, Kajang, Selangor.
³Department of Mechanical, Materials and Manufacturing Engineering, University of Nottingham Malaysia Campus, Selangor

Abstract

Bone tissue engineering requires a biomaterial as a scaffold that plays an essential role to guide new tissue growth in vivo and in vitro model. In designing and developing 3D scaffold for bone tissue engineering the biomaterial’s mechanical strength, fully interconnected porous network, controllable hydrophilicity, degradability, bioactivity and biocompatibility are considered to be important parameters. This study investigated the biocompatibility of biomaterial using osteogenic cell morphology and attachment behavior during in vitro culture expansion.

Keywords

Tissue Engineering, Bone, Biomaterials, Osteogenic cells, Biocompatibility

1.0 Introduction

Tissue engineering (TE) requires a mechanically stable, biocompatible and biodegradable scaffold that allows cell adhesion, cell proliferation, cell specific properties preservation, and suitable for surgical implantations (1). Over the past decade, the main goal of bone tissue engineering has been to develop biomaterials as bone graft substitutes for filling large bone defects (2). Ideally, a bone graft should be porous, be able to stimulate new bone formation, and should possess mechanical properties matching with human bone (3). In vitro cell culture study has the advantage of relatively well-controlled variables, and is generally accepted as a very effective method for biocompatibility testing; the sensitivity is equal or greater than that of in vivo studies (4).

2.0 Materials and Method

2.1 Cell culture on scaffolds and phase contrast imaging

Osteogenic-induced bone marrow derived stem cells (OISCs) were obtained from Tissue Engineering Centre, UKMMC, Cheras, Kuala Lumpur, Malaysia. Two types of poly-lactic acid + hydroxyapatite (PLA+HA) scaffolds fabricated using different stabilizers were provided by the Department of Materials Engineering, Universiti Tenaga Nasional, Malaysia. Approximately 5x10⁵ OISCs were seeded onto each type of scaffold material of 0.5x0.5cm² in a 24-well plate. The seeded scaffolds were immersed in DMEM/F12 (GIBCO, Invitrogen Co., NY, and USA) supplemented with 10% fetal bovine serum (FBS; GIBCO). During the culture period, the cells were incubated at 37⁰C in a humidified atmosphere of 5%
CO\textsubscript{2} and 95\% air and the medium was changed every alternate day. Morphology and attachment behavior of the cells surrounding the scaffold were observed daily using inverted microscope under phase contrast (CK40; Olympus, Japan). On day 7 of culture, cell-seeded scaffolds were processed for scanning electron microscope imaging.

2.2 Scanning Electron Microscopy (SEM)

Scaffolds seeded with cells were preserved in 4\% gluteraldehyde (GA) at 4\°C overnight, followed by dehydration using gradient alcohol solutions of 35\%, 50\%, 70\% and 90\% concentrations, respectively for 30 minutes. The dehydration process ended with 100\% alcohol for one hour and repeated three times followed by drying using the Critical Point Dryer machine (CPD). This time, the drying process involved a solution of acetone and carbon dioxide gas. Following that, the constructs were coated with gold using a sputter coater machine and viewed with a scanning electron microscope (Phenom G2 Pro, Netherlands).

3.0 Results

Cell morphology and cell attachment behavior evaluation via phase contrast imaging was complimented with the observation using scanning electron microscopy. Phase contrast imaging revealed the health state of the cells surrounding the scaffold while scanning electron microscopy revealed the health state of the cells on (in direct contact with) the scaffold. Differential cell morphology and cell attachment behavior were noted on the two types of PLA+HA. For the first type, the surrounding cells appeared attached and well spread on the culture plate. Well-attached and spread cells were also found in abundance on the surface of the scaffold. This indicated that the scaffold is compatible with the osteogenic cells. For the second type, both the surrounding cells and those found on the scaffold surface appeared scarce, round and disintegrating. This indicated that the leachate from the scaffold and the scaffold itself is toxic to the cells.

4.0 Discussions & Conclusions

An ideal biomaterial for bone tissue engineering would administer the appropriate signals to direct the processes of osteogenesis, such as cell attachment, proliferation, differentiation, matrix deposition and ultimately mineralization of extracellular matrix. This study conducted using osteogenic-induced cells shows that the biocompatibility of the materials using the first type of PLA/HA is better compared to the second type PLA/HA based on cell morphology and cell attachment behavior. This method provides a simple screening approach for the determination of material biocompatibility.

Acknowledgement

The study was funded by UNITEN research grant (J 510050374), UKM fundamental grant (FF-2014-129) and Tissue Engineering Research Group Grant: DPP-2014-12.

References


<table>
<thead>
<tr>
<th>BIOMATERIAL</th>
<th>Inverted Optical Microscope (IOM)</th>
<th>Scanning Electron Microscope (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st condition of PLA + HA</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>2nd condition of PLA + HA</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Fig. 1 Inverted Optical Microscopic and Scanning Electron Microscopic Images, 7 days after cell seeding.