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Unmodified medium chain length polyhydroxyalkanoate (uMCL-PHA) as a thin film for tissue engineering application – characterization and in vitro biocompatibility

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ABSTRACT

In the present study, we developed an unmodified/raw Medium chain length –PHA (MCL-PHA) polyhydroxyalkanoate (PHA), natural microbial polyester by using cost-effective saponified palm kernel oil (SPKO) technique. The functional groups, elemental composition, phase purity, water contact angle, and *in* vitro human-derived mesenchymal stromal cell attachment were examined. FTIR confirmed the presence of functional groups corresponding to alkyl halide, alkyne, hydroxyl group, and alkane groups, while XRD and EDX results revealed its phase purity and presence of elements such as carbon and oxygen respectively. SEM and confocal microscope analyses revealed that the bio-material supports cell attachment and this was further confirmed through cell viability assay. In conclusion, the characterization and compatibility studies revealed that this novel scaffold could be a potential candidate for possible tissue engineering applications.

1. Introduction

The identification and development of biomaterials is an indispensable process required for the progression of novel therapeutics. Many biomaterials are being designed and then individually tested for their properties by combining these materials with one or more specific cell type. The aim is to find the appropriate use of a material for a particular application with the emphasis made for bio-compatibility and minimal immune reaction [1]. Many biomaterials are used routinely and provide an ever important role in the treatment of diseases [2]. However, an ideal scaffold/carrier either natural or synthetic must possess chemical stability or degradability. In addition, these materials should have physical properties matching the surrounding tissue in order to provide cytocompatibility, support adhesion, proliferation, stability, and mechanical strength.

Polyhydroxyalkanoate (PHA), a group of naturally occurring biodegradable and biocompatible versatile polyesters produced by many bacterial species as intracellular storage compounds is considered to be one such biomaterial [3]. Even though medium chained length polyhydroxyalkanoate (MCL-PHA) comprising 6–14 molecules of carbon sequence was generally used in medical field, reports have summarized that all MCL-PHAs were co-polymerized (with different side chains), in order to make it more malleable. MCL-PHA copolymers can be produced using a variety of substrates including plant oils. Due to their long carbon number, these substrates have high energy content which is excellent for good cell growth and energy metabolism. Preliminary studies on the production of MCL-PHA from renewable and cost-effective substrates such as palm oil, palm kernel oil, saponified palm kernel oil (SPKO) and other major fatty acids fractions by *Pseudomonas putida* have also been reported [4]. However, the product obtained following co-polymerization has reported several issues including material toxicity during degradation [2], thus increasing the need for unmodified/raw MCL-PHA (without any co-polymerization). Unfortunately, studies on unmodified/raw MCL-PHAs (uMCL-PHA) as a potential biomaterial have yet to be demonstrated in any known reports. Therefore, a study was conducted to characterize and optimize MCL-PHA (produced from SPKO, without any co-polymer composition) and to determine the adhesion and proliferation potential of Mesenchymal stromal cell (MSCs) on this biomaterial. Amnion (AM), a readily available, thin, highly flexible biocompatible/biodegradable material, obtained during child birth

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was processed and was used as reference standard in this study. Previous studies have demonstrated its use in various medical applications, such as wound dressing, tissue adhesion barriers, and ophthalmologic surgery. Studies have demonstrated that AM exhibits anti-adhesive, anti-inflammatory, and antimicrobial properties [5].

2. Materials and methods

uMCL-PHA Preparation: uMCL-PHA was prepared using SPKO as described earlier [4]. Briefly, 8 g of Palm kernel oil (PKO) from the nut of *Elaeis guineensis Jacq*. fruit was added to the ethanolic (100 ml) potassium hydroxide solution (2.8 g). The mixture was refluxed (1hr) and the ethanol was removed, leaving behind the solid potassium salts of fatty acids. Experiments were conducted using rotary shaking flasks at 240 rpm, 30 °C. *Pseudomonas putida* cells were grown in E medium supplemented with 0.1% microelement solution to produce the biomass. After 20hrs the cells were harvested, washed and transferred to the nitrogen-limiting E2 medium containing a specified carbon substrate at a concentration of 0.5% (w/v). Cells were harvested after 48 h by centrifugation, washed and lyophilized. Lyophilized cells (0.5 g) were suspended in 100 ml chloroform and refluxed for 4 h. The mixture was filtered and concentrated by evaporation. The polymer was precipitated in methanol and dried in a vacuum oven at 50 °C as a thin sheet.

Characterization of uMCL-PHA films: The materials were characterized using Fourier-transform infra red spectroscopy (FTIR; JSMIFS 66v/s Bruker,USA), X-ray diffraction (XRD; D8Bruker-AXS,USA), scanning electron microscopy (SEM; JSM6400; JEOL, Japan), energy-dispersive X-ray spectroscopy (EDS; INCAEnergy200, Oxford Inst.), and confocal microscopy (Leica TCS-SP5 II, Leica Microsystem, Germany). The surface wettability of the films was measured using a contact angle goniometer (Model G-1 type; Erma Inc., Japan).

Cell Culture: Human Mesenchymal stromal cell (hMSCs) suspension with cell density of 2×10^4 cells/cm^2 was seeded on to the sterilized (gamma irradiated) uMCL-PHA ($n=5$) and amnion ($n=5$) placed in a 6-well plates (ultra low attachment plates). The same density of cells was also seeded onto the normal coated six-well tissue culture plates. The specimens were cultivated in 3 mL of culture media (DMEM) supplemented with 10% FBS, 100 U mL^{-1} of penicillin and 100 μg mL^{-1}of streptomycin and 1% of glutamine for nourishment. Then these specimens were placed in a humidified incubator supplied with 5% carbon dioxide at 37 °C, and analyzed at days three and six. uMCL-PHA and amnion without cells was used as the control.

Cell proliferation: Cell proliferation was determined using Alamar blue assay kit (Life technologies, US). Statistical analyses were

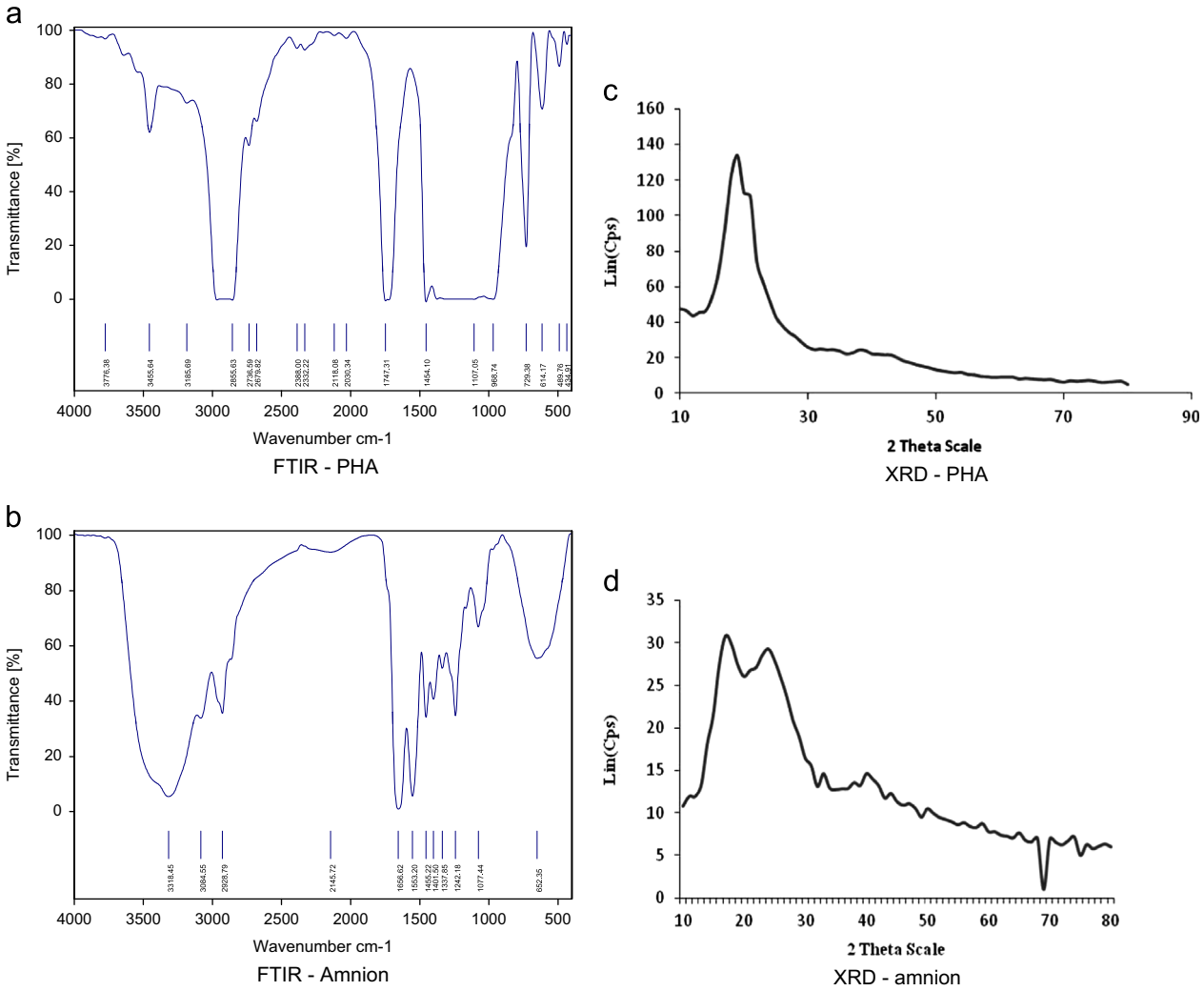

Fig. 1. The functional groups of MCL PHA and amnion using FTIR (a and b). XRD pattern of MCL-PHA and amnion (c and d).