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Cell colonization potential of thermoplastic silicone-based polyurethane nonwovens for novel heart valve prosthesis

Abstract: Heart valve tissue engineering aims at creating living valves through colonization of scaffolds with patient's own cells. Various cell sources have been explored focusing mainly on endothelialization of the scaffold surface. Endothelial like cells, such as endothelial progenitor cells (EPCs), which can be isolated from peripheral blood or bone marrow could be a suitable option. In this study we investigated cell colonization potential of ovine EPCs (OEPCs) on thermoplastic silicone-based polyurethane (TSPU) polymer scaffolds. TSPU nonwovens with and without vascular endothelial growth factor (VEGF) functionalization were used. SEM images showed that by day 3 the cells were growing as patches on the surface of both polymer groups. The cell patches continued growing and started covering more of the nonwoven surface. On day 7 the cells had almost covered the scaffold surface. The cells were more uniformly distributed as monolayer on the functionalized TSPU compared to the non-functionalized nonwovens. Live/Dead staining provided bright green fluorescence on the samples, indicating metabolically active cells. These static cell seeding experiments demonstrated that functionalized TSPU nonwovens support endothelialization. The feasibility of TSPU nonwovens as heart valve prosthesis scaffold could be further explored with animal studies in an ovine model.

Keywords: Heart valve scaffolds, nonwoven polymers, endothelial progenitor cells.

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1 Introduction

Heart valve prosthesis engineering aims at creating living valves through colonization of suitable scaffolds with patient's own cells. The process of cellular incorporation into the scaffolds could be prepared *in vitro* before implantation or it could be achieved *in vivo* inside the patient's body after implantation [1]. For the *in vitro* approach various cell sources have been explored focusing mainly on endothelialization of the scaffold surface. Several studies have been performed which investigated isolation of endothelial cells by enzymatic digestion of blood vessels, but this requires sacrifice of healthy blood vessels. As an alternative, researchers are also investigating to utilize endothelial like cells, such as endothelial progenitor cells (EPCs), which can be isolated from peripheral blood or bone marrow [2].

In this study, we used ovine endothelial progenitor cells (OEPCs) with the future prospect of implanting polymeric heart valves in sheep for animal experiments. These OEPCs were isolated from ovine blood and their colonization potential on decellularized scaffolds have already been studied in our research group. Thermoplastic silicone-based polyurethane (TSPU) polymer scaffold was utilized as the scaffold material. Silicone based polyurethane have been tested as heart valve leaflet material [3] and could serve the purpose better following endothelialization. Thus, the objective of this phase of the study was to investigate the colonization potential of OEPCs on thermoplastic silicone-based polyurethane polymer under static seeding condition.

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2 Materials and methods

2.1 Culture and expansion of ovine endothelial progenitor cells

Ovine endothelial progenitor cells were cultured in Nunc cell culture flask containing endothelial growth medium supplemented with 1% (v/v) penicillin streptomycin. The growth medium was prepared by mixing the contents of EGM-2 SingleQuots™ (Lonza) kit into the Endothelial Cell Basal Medium-2 (EBM-2, Lonza). The cells were cultured at 37°C, 5% CO₂ atmosphere and 85% humidity (see Figure 1). The cells were passaged upon confluence and then seeded onto the polymer at passage three.

Figure 1: OEPCs cultured and expanded in culture flask at passage three.

2.2 Polymer samples

Functionalized electrospun polymer samples (functionalized TSPU) were utilized for static cell seeding experiments. For functionalization, the polymer was first plasma treated, then sterilized with 70% ethanol for seven minutes and after that functionalized with vascular endothelial growth factor (VEGF) under sterile conditions. For comparison, non-functionalized polymer (without plasma treatment and without VEGF coupling), identified as non-functionalized TSPU, was used. The non-functionalized polymer was prepared under non aseptic condition. Each of the polymer samples had a size of 2x2 cm².

2.3 Sterilization

Prior to the cell seeding experiment, the non-functionalized TSPU were sterilized with 0.1% peracetic acid (pH 7.3) treatment. The samples were immersed into peracetic acid and incubated for three hours on an orbital shaker. Then the samples were rinsed six times with sterile PBS for 30 minutes per wash.

2.4 Cell seeding experiment

Static cell seeding experiments were carried out in 6-well plates. The 2x2 cm² polymer samples were placed into the wells. Metal rings with a radius of 0.6 cm were placed above each of the polymer samples. OEPCs were suspended in 230 µl culture media and added on the polymer, inside the metal rings (see Figure 2). OEPCs were seeded at the density of 5×10^4 cell/cm². The seeded scaffolds were then cultured at 37 °C, at 5% CO₂ and 85% humidity, for two time points: 3 and 7 days. For each time point there were three samples. As a positive control, cells were cultured on wells without polymer. For negative control, polymer samples were incubated on the wells together with cell culture media without cells. The cell culture medium was changed every day till the last time point.

The samples were examined through Live/Dead staining (ThermoFisher Scientific, Cat. No. L3224) to check the viability of the cells. The progression of cellular colonization on the polymer surface was visualized through Scanning electron microscopy (SEM).

Figure 2: Experimental setup for static cell seeding of polymer.

3 Results

Cells seeded on the polymer surface were clearly visible on the SEM images. On day 3, the cells could be observed as patches on surface but didn't cover the entire surface (see Figure 3). As the days progressed the area of the cell patches increased and started occupying more of the polymer surface. By day 7 the cells had almost filled the polymer surface. Interestingly, at day 7 difference could be observed in the colonization pattern of cells between the two polymer groups. In case of functionalized TSPU, the underlying fiber pattern of the polymer could be observed on the cell sheet covering the polymer indicating that the cells were attaching firmly to the functionalized polymer. This feature was not seen for the non-functionalized group.

Figure 3: SEM images of polymer surface seeded with cells and control polymer surface without cell seeding.

Bright green fluorescence was observed on samples that underwent Live/Dead staining, indicating presence of live cells. Only few red spots, representing dead cells, were seen. The same was observed for the positive controls (see Figure 4). In case of negative control, containing just polymer without cell seeding, there was neither green nor red signal.

Figure 4: Figure 3: Live/Dead staining revealed cells were growing on polymer and well plate surface (positive control).

4 Conclusion

The static cell seeding experiments performed in this phase showed that OEPCs were able to attach and grow on the surface of TSPU polymer. The study revealed that cells were alive and proliferating after up to 7 days of culture on the polymer surface. These static cell seeding experiments demonstrated that functionalized TSPU nonwovens support endothelialization. Therefore, the feasibility of TSPU nonwovens as heart valve prosthesis scaffold could be further explored with animal studies in an ovine model.

Author Statement

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