CELL ADHESION TO PORCINE ELASTIN BIOMATERIALS

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ABSTRACT

We have investigated the initial adhesion and subsequent growth rate of three cell types to porcine elastin biomaterials (PEBs). Porcine Aortic Smooth Muscle Cells (PASMC), Primary Porcine Adipose-Derived Stromal Cells (PADSC), and Porcine Aortic Endothelial Cells (PAEC) were seeded onto both the PEB and controls of tissue culture plastic (Thermanox) for timepoints ranging from 2 hours to 2 weeks. Direct fluorescent staining was used to label cell nuclei and F-actin fibers, while elastin autofluorescence was used to simultaneously visualize the PEB surface structure. PASMCs adhered quickly to the PEB, yet all cell types had slower growth rates compared to the controls (3.09 vs 9.27 cells/mm^2/hour for PASMC, 1.73 vs 3.76 cells/mm^2/hour for PADSC and 4.24 vs 7.24 cells/mm^2/hour for PAECs) for the first 48 hours. The concentration of cells was similar by 1 week. Cell morphology markedly changed from rounded cells (2 to 6 hours) to spread with dense and aligned central F-actin stress fibers at 24 to 72 hours. The PASMCs aligned along the longitudinal elastin fibers of the PEBs. We conclude that elastin biomaterials provide a favorable substrate for cell growth and attachment and that cell morphology is markedly influenced by elastin biomaterial structure.

MATERIALS AND METHODS

Elastin Isolation

Porcine carotid arteries were obtained from adult swine of (Animal Technologies, Tyler, TX). The arteries were transported overnight in cold phosphate buffered saline (PBS), and the gross fat was dissected away. Using aseptic techniques, the arteries were then sequentially treated with 80% ethanol (minimum 72 hours, 4°C), 0.5M NaOH for 75 minutes with sonication at 60^{o}C, and two 30 minute room temperature washes in HEPES buffer (pH 7.4). The extracted porcine elastin biomaterials (PEBs) were then stored in fresh HEPES, autoclaved at 121^{o}C for 15 minutes, and stored at 4^{o}C in HEPES.

Cell Types

Three types of cells were used: Primary Porcine Aortic Smooth Muscle Cells (PASMC), Primary Porcine Adipose-Derived Stromal Cells (PADSC), and Porcine Aortic Endothelial Cells (PAEC, Clonetics). Cells were seeded onto the PEBs at passage 4 or below. PASMC and PADSC were isolated directly from porcine tissues.

Adhesion Studies

Coverslips of tissue culture plastic (Thermanox) were used as the control materials. PEBs were secured lumen side up with PharMed tubing (OD = 7/8", ID = 5/8") in 12-well culture plates and hydrated with PBS in the incubator (37^{o}C, 5%CO_2/95%Air). Each 12-well plate had triplicate wells of 3 experimental conditions and 1 control condition. All wells were seeded at 2.3 x 10^4 cells/cm^2 and returned to the incubator. Cells were allowed to adhere and grow for 2, 6, 24, 48, 72 hours, 1 week and 2 weeks. At the designated timepoints the cells were rinsed 2X with PBS and fixed with 2% paraformaldehyde.

Analysis of Adhesive Cells

Cell nuclei were stained to determine the number of adherent cells, while F-actin staining determined cell morphology. Serial dilutions of all stains were performed to determine the optimal concentration for use with PEBs. Histology and Scanning Electron Microscopy (SEM) analysis provided information about cell penetration into the biomaterials and comparison to traditional enface techniques, respectively. Each PEB material was sectioned into quadrants with (A) stained cell nuclei with SYTOX® Green (Molecular Probes, Eugene, OR), (B) stained F-actin with Alexa Fluor® 568 phalloidin (Molecular Probes, Eugene, OR), (C) sectioned for histology (Portland Tissue Processing, Portland, OR) with Hematoxylin and Eosin staining, and (D) SEM analysis. Cell counts were performed on four random positions of the SYTOX stained specimen using ImageJ software. The phalloidin stained specimens were imaged with a Radiance Confocal Microscope (BioRad). PEB autofluorescence allowed the investigation of the cell morphology in relation to the elastin biomaterials.

RESULTS

The number of adherent cells two hours after innoculation is shown in Figure 1, and the cell growth curves are shown in Figure 2. PASMCs rapidly adhered to the PEB and had a slower growth rate

compared to the tissue culture plastic controls with the number of cells plateauing between 1 and 2 weeks. Histology indicated that there was no cell penetration into the elastin after 48 hours (data not shown).

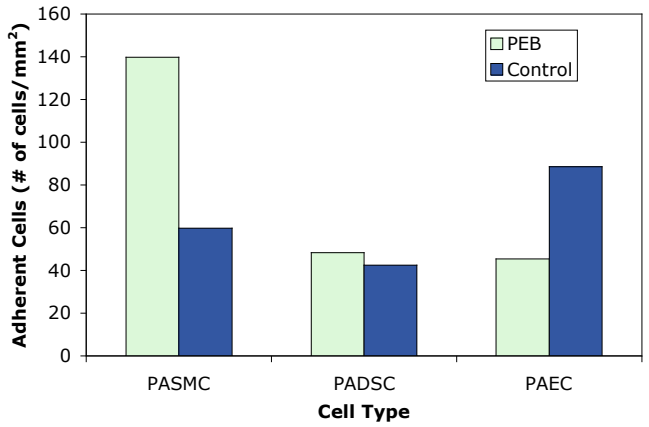

Figure 1: Adhesion of cells 2 hours after innoculation.

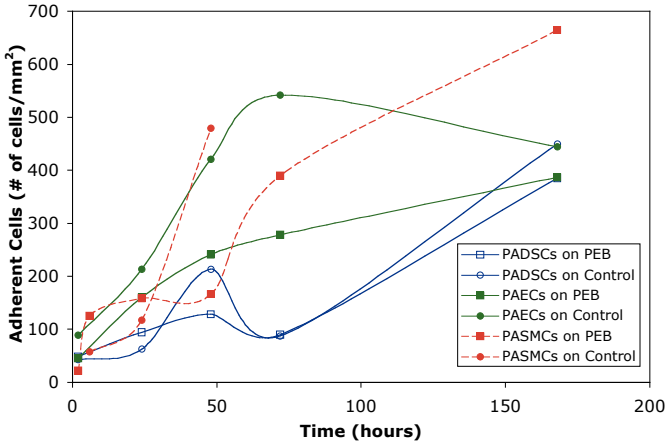

Figure 2: Adhesion of PADSCs and PAECs to PEBs and controls up to 1 week.

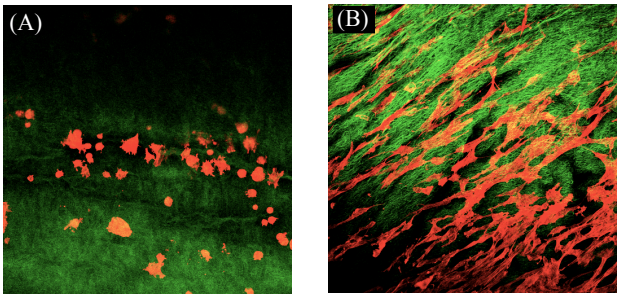

Figure 3: F-actin staining of adherent PASMCs on autofluorescent PEBs at (A) 2 hours and (B) 72 hours (10X objective).

From 2 to 48 hours, PADSCs adhered to PEBs and controls at a slower rate (1.73 and 3.76 cells/mm^2/hour) compared to PAECs (4.24 and 7.24 cells/mm^2/hour), respectively. PASMCs had adherence rates to PEB and controls of 3.09 and 9.27 cells/mm^2/hour, respectively, similar to PAEC adherence rates. While these cell types grew at a slower rate on the PEBs compared to the controls, the concentrations of cells were similar after one week.

After 2 hours of incubation all cell types were loosely attached with a rounded configuration (Figures 3(A) and 4(A)). By 24 hours cell concentrations on PEB were increasing and aligned F-actin stress fibers had appeared (data not shown). At 72 hours cells were firmly adherent with F-actin fibers strongly oriented in the direction of PEB surface fibers (Figures 3(B) and 4(B)). The overall orientation of the cells also showed that PASMCs tended to attach and grow along the longitudinal fibers of the PEBs (Figure 5).

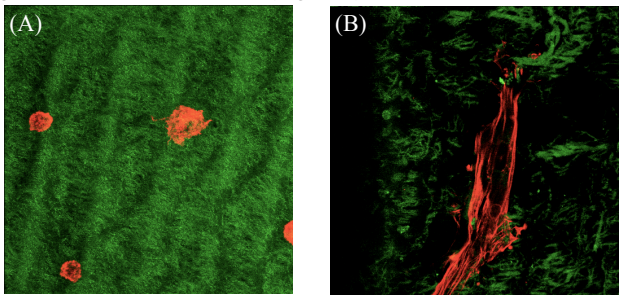

Figure 4: F-actin staining of adherent PADSCs on autofluorescent PEBs at (A) 2 hours (10X objective) and (B) 72 hours (60X objective).

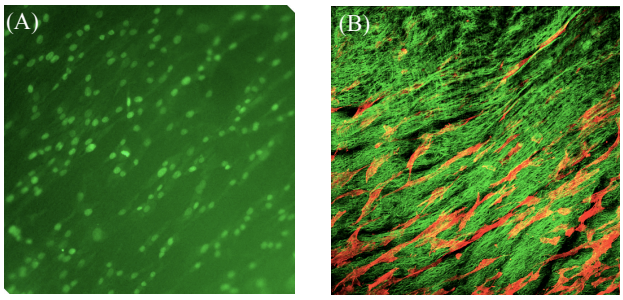

Figure 5: Adherent PASMCs on autofluorescent PEBs at 72 hours (A) SYTOX nuclear stain and (B) F-actin stain showing alignment of cells along elastin fibers in the longitudinal axis of the material (10X objective).

DISCUSSION AND CONCLUSIONS

Endothelial and smooth muscle cells adhered at slower rates compared to tissue culture plastic controls, but were equivalent by 1 week. The PASMCs attached and grew in the direction of the elastin fibers, indicating a significant effect of matrix structure on cell morphology. The PASMC morphology with a pronounced long axis was significantly different than the PAEC morphology without a predominant long axis, classical cobblestone appearance. The dip in number of PADSC cells at 72 hours could indicate the selective growth of a specific phenotype possibly driven by the adherence characteristics of the elastin biomaterial. These fluorescent techniques can now be used to further study cell adhesion to elastin including focal contacts and binding mechanisms.

ACKNOWLEDGEMENTS

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