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TEXAS MEDICAL CENTER NASA/JOHNSON SPACE CENTER
COOPERATIVE AGREEMENT NCC9-36
Applications Subcontract Program, Round 2

<<< Final Report >>>

Baylor College of Medicine
Charles L. Seidel, Ph.D.
"Interaction of Vascular Smooth Muscle Cells Under Low Shear Stress"

Background

The blood vessel wall consists of three cellular layers, an outer adventitial, a middle medial and an inner intimal layer. When the blood vessel forms in the embryo it begins as a tube composed of a single cell type called endothelial cells. Over time, other cells are recruited from the surrounding tissue to form additional layers on the outer surface of the endothelial tube. The cells that are recruited are called mesenchymal cells. Mesenchymal cells are responsible for the production of connective tissue that holds the blood vessel together and for developing into vascular smooth muscle cells that are responsible for regulating the diameter of the vessel (1) and therefore, blood flow. In a fully developed blood vessel, the endothelial cells make-up the majority of cells in the intimal layer while the mesenchymal cells make-up the majority of cells in the medial and adventitial layers. Within the medial layer of a mature vessel, cells are organized into multiple circular layers of alternating bands of connective tissue and cells. The cell layer is composed of a mixture of mesenchymal cells that have not developed into smooth muscle cells and fully developed smooth muscle cells (2).

The assembly and organization of complex tissues is directed in part by a signaling system composed of proteins on the cell surface called adhesion molecules. Adhesion molecules enable cells to recognize each other as well as the composition of the connective tissue in which they reside (3). It was hypothesized that the different cell types that compose the vascular wall possess different adhesion molecules that enable them to recognize each other and through this recognition system, form the complex layered organization of the vascular wall. In other words, the layered organization is an intrinsic property of the cells. If this hypothesis is correct then the different cells that make up the vessel wall, when mixed together, should organize themselves into a layered structure resembling an intact blood vessel. Experiments described below were designed to test this hypothesis.

We have identified and developed a technique to isolate two cell populations from the medial layer of blood vessels that enable us to study the interaction of these distinct cell types when combined in cell culture (4). One cell type, the vascular smooth

muscle cell (VSMC) has characteristics of a fully developed muscle cell. The other cell type, the Type 2 cell, has characteristics of a mesenchymal cell that has not developed into a muscle cell. Using these two cell populations we set about to determine if they are capable of segregating and assembling into a layered structure in culture. To best imitate conditions in the body during blood vessel assembly where cells are able to move in all three dimensions, we made use of a special cell culture system developed at NASA, the rotating cell culture system (RCCS). The design of the RCCS is such that cells are suspended motionless in a nutritive media enabling them to interact and form large three-dimensional complexes. To provide a surface for cell growth, small (microcarrier) beads coated with collagen are included in the RCCS. The RCCS has been used to culture several cell types but this is the first time that the RCCS has been used to study the interaction of vascular smooth muscle cells. Initial studies were directed at determining if multicellular complexes could be formed. Having established this, these complexes were examined using electron microscopy to determine if VSMCs and Type 2 cells spontaneously associated into identifiable cell layers within the complexes. The results of these studies are summarized below.

VSMCs and Type 2 cells were maintained in the RCCS for up to twenty days and samples removed at specific intervals for microscopic examination. Within two days macroscopic complexes begin to form. The complexes consist of several collagen coated microcarrier beads held together by vascular cells. It was the organization of these cell complexes that was further investigated by electron microscopy. Figure 1 is a photomicrograph taken three days after culture illustrating the different cell morphologies present in the macrocellular complexes. The large cell in the micrograph is a VSMC while the others are Type 2 cells. These classifications are based on relative cell size, density of the cytoplasm and the presence of a basement membrane. VSMCs are large cells surrounded by a basement membrane with a cytoplasm dense with contractile filaments. Type 2 cells do not have any of these characteristics. By examining such cell complexes at different times in culture four new observations were made.

The first observation was that a large amount of connective material, the material in which cells are imbedded in an intact vessel, was associated primarily with VSMC cells rather than Type 2 cells. Figure 2 is a photomicrograph of a typical VSMC and the associated connective material. The banded appearance of the connective material identifies it as collagen. Collagen can be seen emanating from invaginations of the cell surface where it appears less organized than it is further from the cell surface. It is known that collagen is secreted by cells in a pro-form and is subsequently organized into highly structured strands outside of the cell. These images suggest that the VSMC is responsible for collagen synthesis, secretion, and assembly into fibers. The absence of collagen in association with Type 2 cells suggests that they may not be involved in its synthesis.

The second observation was that Type 2 cells, but not VSMC were observed to make intercellular connections. No intercellular connections were observed between

Type 2 cells and VSMC or between individual VSMCs. Type 2 cells were observed to form two types of intercellular connections, adherens junctions and gap junctions. These are illustrated in Figure 3. Adherens junctions enable two cells to be physically interconnected through a protein called, cadherin. Such physical interconnections provide structural support so that individual cells can form organized tissues. As described below, Type 2 cells form overlapping cell layers which may be coupled by adherens junctions. Gap junctions, on the other hand, not only provide physical intercellular connections through the connexin protein but also permit intercellular communication through direct cytoplasmic contact. Gap junctions permit electrical and chemical communication between cells enabling groups of cells to act in a coordinated manner. The presence of gap junctions in Type 2 cells implies that they possess this capability.

The third observation was that Type 2 cells, but not VSMC formed overlapping cell layers. As illustrated in Figure 4 these layers were several cells thick and followed the curvature of the microcarrier beads upon which they developed. The absence of VSMC from such layers suggests that these layers do not result from the random association of cells but that cellular segregation occurs. The cause of this segregation is not known at this time but most likely results from the expression of adhesion molecules on the cell surface that allow specific cells to associate with the exclusion of others. The characterization of these molecules on VSMC and Type 2 cells awaits further experimentation. However, this observation suggests that the structural organization of the vessel wall is directed in part by the intrinsic properties of the cells.

The final observation was that Type 2 cells exhibited the ability to phagocytize, that is, digest VSMCs. Figure 5 shows a Type 2 cell completely surrounding a VSMC whose cytoplasm no longer is dense with contractile filaments. We have not determined if Type 2 cells initiated the degradation of VSMCs or if Type 2 cells are digesting VSMCs that were already deteriorating for other reasons. However, over time in culture Type 2 cells become the dominant cell type. This is the first report of phagocytic activity by Type 2 cells and points out a new function for these cells within the vessel. It is possible that following vascular injury and associated cell damage, Type 2 cells may be involved in removing tissue debris thereby facilitating the healing process.

In conclusion, the objective of this research was to determine if VSMCs and Type 2 cells self-assembled into layers similar to those observed in intact vessels. We observed that Type 2 cells, but not VSMCs could assemble into such layers. Such self-assembly may result in part from the ability of Type 2 cells to form intercellular connections, which were not observed between VSMCs. The observation that the cell layers consisted of a homogeneous cell population (i.e. Type 2 cells) rather than a mixture of cells implies that cells can distinguish between one another. This ability would enable cells to form the layered structure observed in intact blood vessels. Results from this study are being prepared for publication.

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Fig. 1 --- VSMC and Type 2 Cells

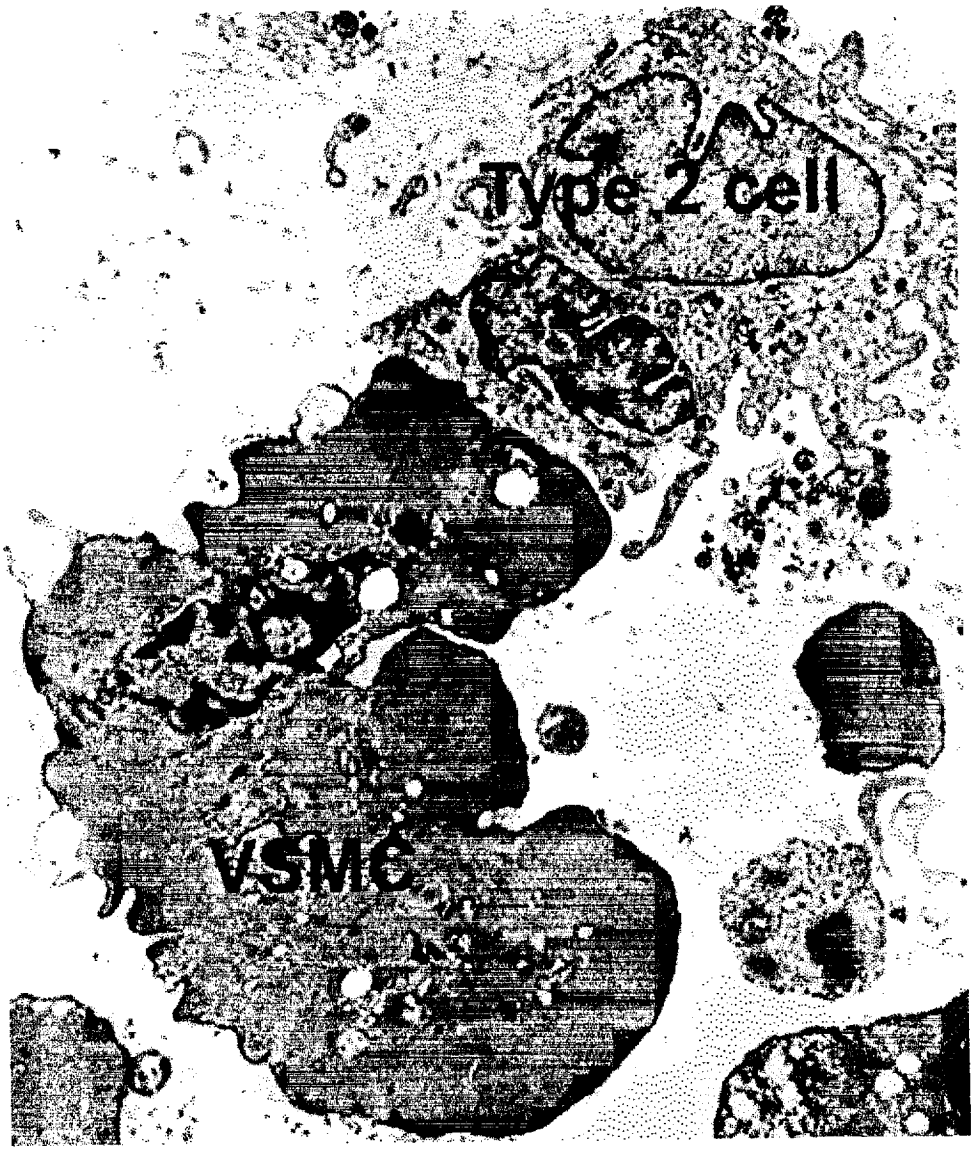


Fig. 2 --- VSMC and Collagen Fibers

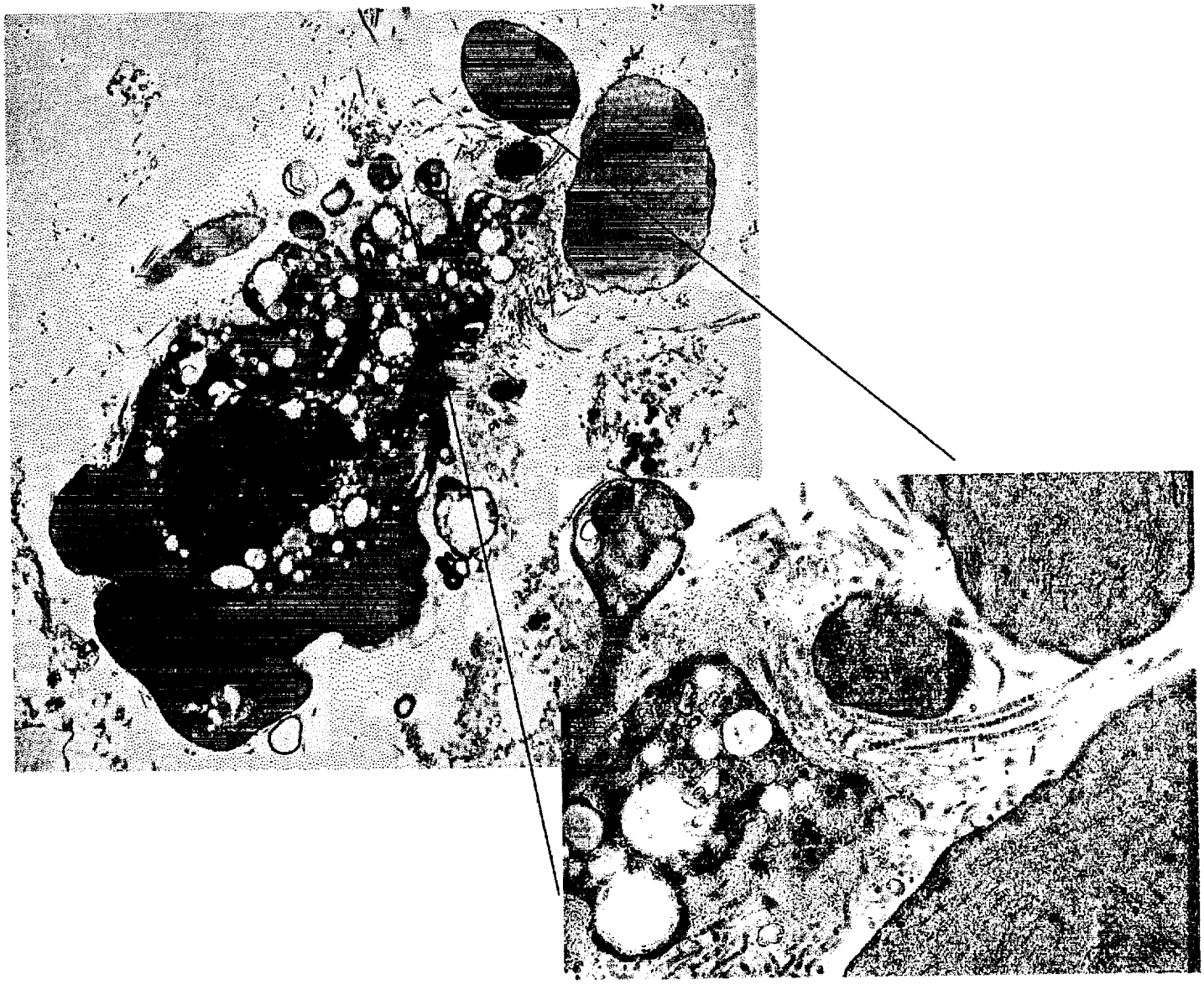
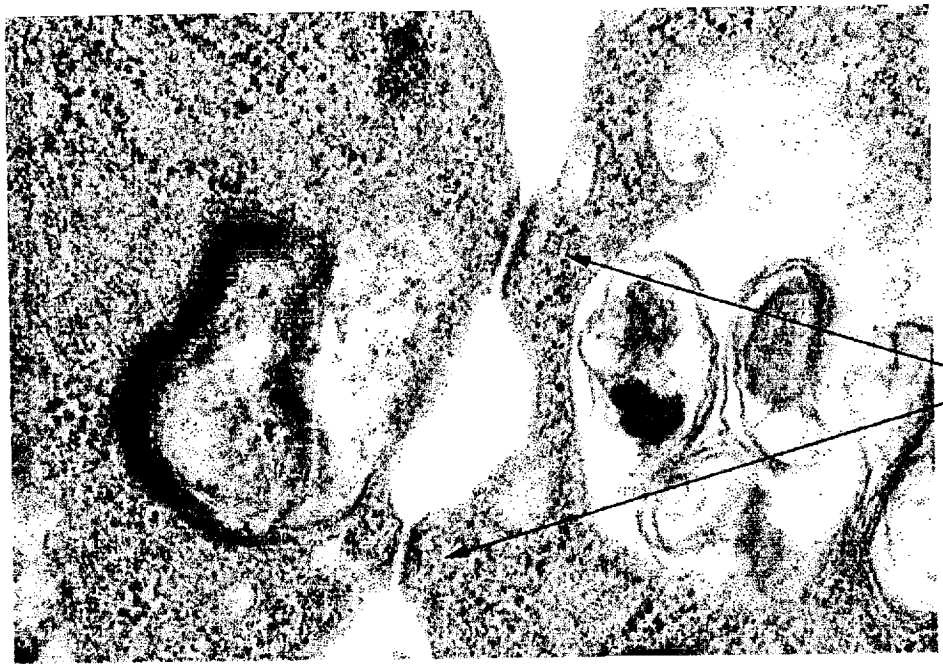


Fig. 3 --- Adherens and Gap Junctions in Type 2 Cells



Adherens Junctions

Gap Junction

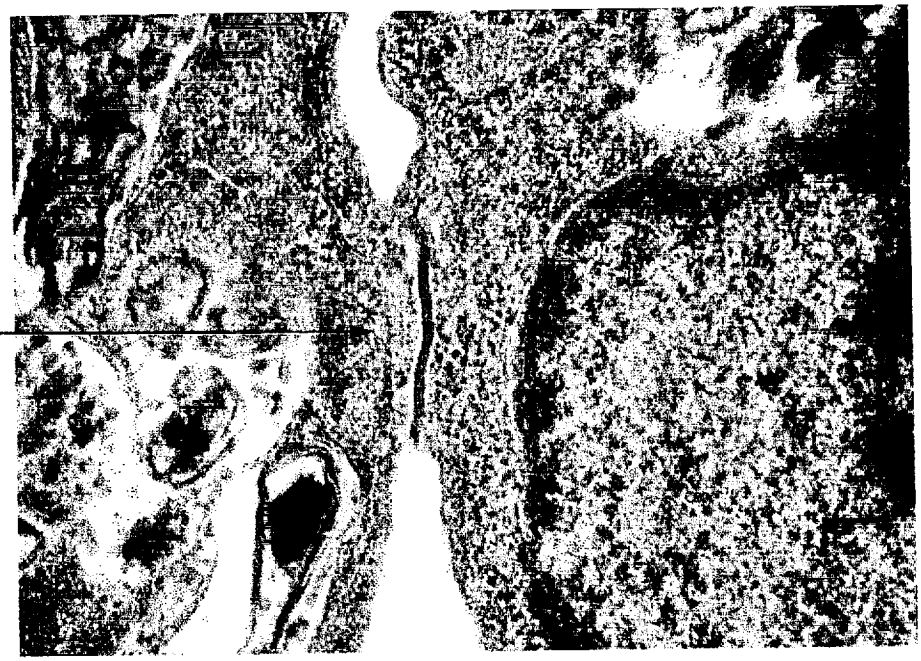


Fig 4 --- Layer Formation by Type 2 Cells

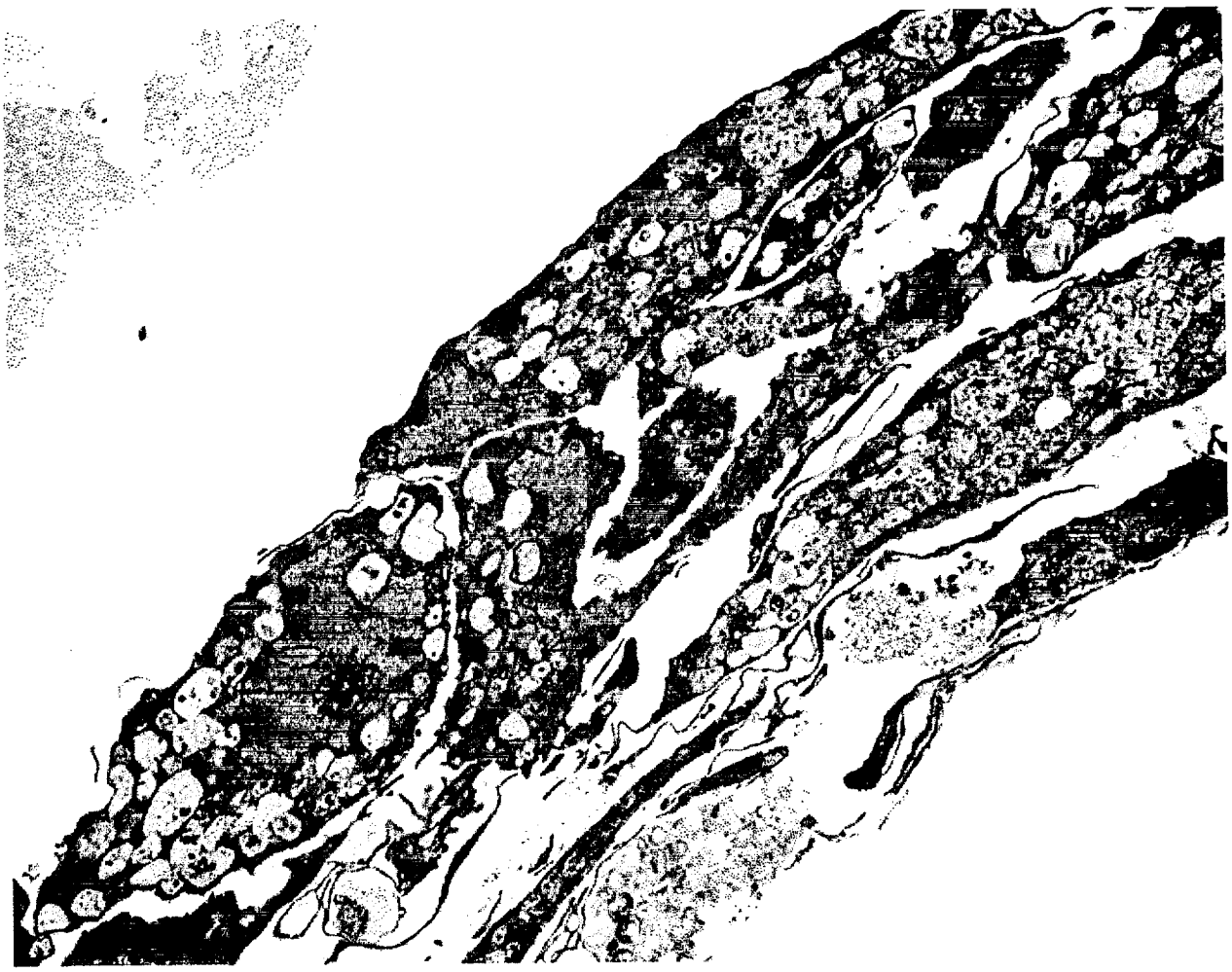


Fig. 5 --- VSMC Being Phagocytized by Type 2 Cell