Experimental Studies of Pulsatile Flow and Endothelial Cell Adaptation in Ventricle Shaped Cell Culture Chambers

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The authors' long-term research goal is to minimize the risk of thromboembolic complications in cardiac prostheses by lining blood contacting surfaces with a functional monolayer of autologous endothelial cells. These cells recognize changes in hemodynamics and can adapt effectively to experimentally manipulated flow conditions. By implication, the morphology of endothelial cells, in conjunction with their function, might serve as an indicator of the flow patterns in a particular location. It was hypothesized that, by understanding flow patterns at a given site, the local morphology and function of the endothelial cells in such a region could be predicted. To test this hypothesis, a series of ventricle shaped flow chambers were designed and perfused with pulsatile flow. The flow field in the chambers was studied by computer aided dye visualization and nuclear scintigraphy. The results showed that the large scale motion of the fluid in the cavity was highly coherent and consisted of distinct flow patterns. The temporal and spatial characteristics of the flow patterns, and their implications with respect to endothelial cell endurance in this *in vitro* environment, were examined in detail. *ASAIO Journal* 1992; 38: M501–M506.

Extended use of cardiac prostheses as a replacement for failing hearts depends, to a large extent, on successful reduction of thromboembolic events that result from the intrinsic thrombogenicity of the synthetic blood contacting surfaces.[1] A promising approach to achieving this goal relies on lining the blood contacting surfaces with a functional monolayer of autologous endothelial cells (ECs). EC morphology and function is closely related to local hemodynamics. In particular, ECs can recognize changes in hemodynamics and adapt effectively to diverse flow conditions. This concept has been demonstrated *in vivo* in experimentally manipulated blood vessels[2] and substantiated *in vitro* in parallel-plate flow chambers[3,4] and cone-and-plate set-ups.[5,6] The inherently unidirectional flow produced by such systems is suitable for studies of EC adaptation in vascular grafts, but it is inadequate to simulate complex flow patterns inside cardiac prostheses. Before testing the attachment and endurance of ECs on the luminal surface of a blood sac in a beating artificial heart, we studied the adaptation of endothelial cells in ventricle shaped cell culture chambers designed to mimic the flow patterns in cardiac prostheses.

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Methods, Results, and Discussion

Cell Adaptation Studies

We recently described the adaptation of a monolayer of human adipose microvascular ECs to nonunidirectional flow inside a flow-through apparatus of the size of a microscope slide.[7] After 24 hrs of perfusion at a flow rate of 50 ml/min (Reynolds number = 540), the EC monolayer showed no signs of denudation in the entire cavity of the cell culture chamber. The alignment of ECs followed the mean flow patterns in distinct areas of the flow field, e.g., the central core of the laminar jet in the impingement zone, and in the area of jet entrainment at the edge of the recirculating flow. Additional detailed examination of the monolayer focused on sites such as the outskirts of the impingement region and in the recirculating vortex (where the flow was disturbed and intermittently turbulent). The orientation of cells in those areas was either random **(Figure 1)** or followed the curvature of the flow in a crisscrossed manner. It differed substantially from the typical cell structure in a locally unidirectional flow. These results showed that microvascular ECs, like their large vessel derived counterparts, can adapt effectively to experimentally manipulated conditions *in vitro* that do not neces-

sarily entail unidirectional or laminar flow if the level of local flow disturbances is low.

We recently examined the endurance of EC monolayers at high Reynolds number flows. In these studies, we used sheep heart endocardial ECs grown to confluence on the fibronectin treated bottom of a flow-through cell culture chamber (FTCCC).[8] After 24 hrs of exposure to flow at 100 ml/min, the EC monolayer remained intact across the inner portion of the circular cavity, including the core region of the central jet (**Figure 2**). However, our studies also showed partially denuded areas that were restricted to the regions of jet impingement and flow recirculation. In the latter case, the denuded sites were not contiguous. In many places, only a few cells were missing. In others, the number of absent ECs was relatively large, indicating the spatial extent of the high level disturbances in these areas. Thus, the map of the EC monolayer at these sites was an indicator of the flow patterns in those locations. We hypothesized that the converse approach would be equally applicable. By knowing the detailed characteristics of the flow patterns in the flow chambers, we can focus on select sites and predict the local morphologic structure and, presumably, the functional state of the ECs in those regions.

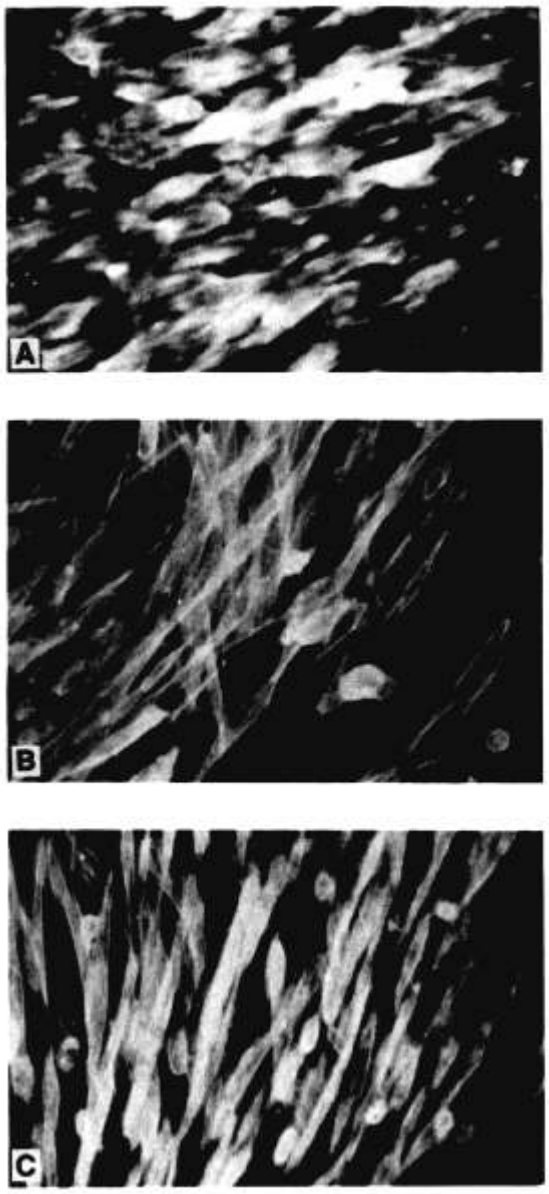


Figure 1. Alignment of human microvascular adipose endothelial cells in various flow regions inside a microscope slide cell culture chamber. (A) Highly disturbed flow. (B) Disturbed flow within the recirculating eddy. (C) Locally unidirectional flow ×200.

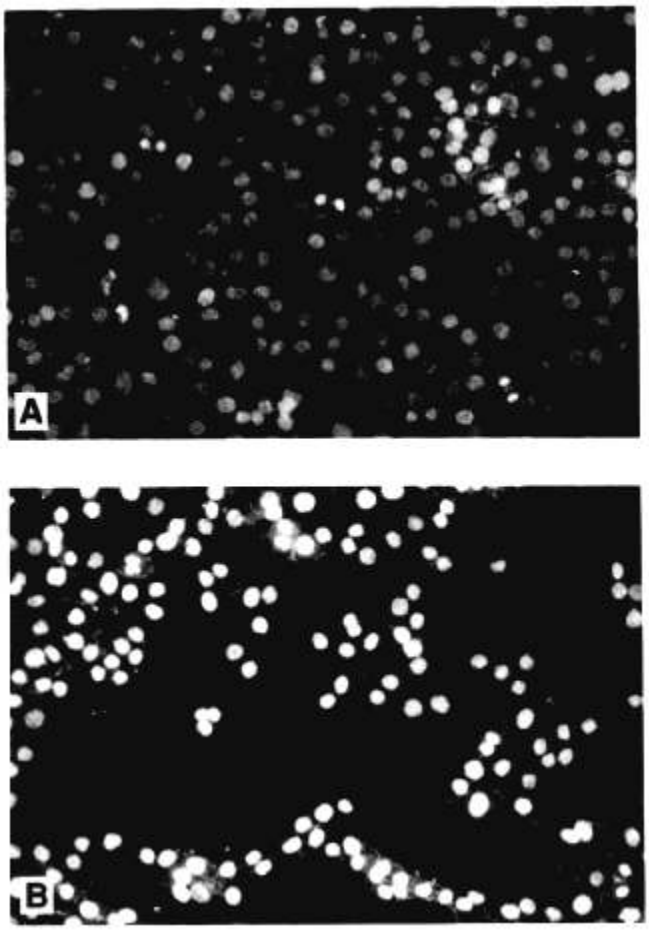


Figure 2. Endurance of a sheep heart endocardial endothelial cell monolayer inside the FTCCC. (A) Core region of central jet. (B) Region of jet impingement. Original magnification ×200.

Flow Pattern Studies

The flow patterns in ventricle-like cell culture chambers were studied using dye visualization, gray-tone image analy-

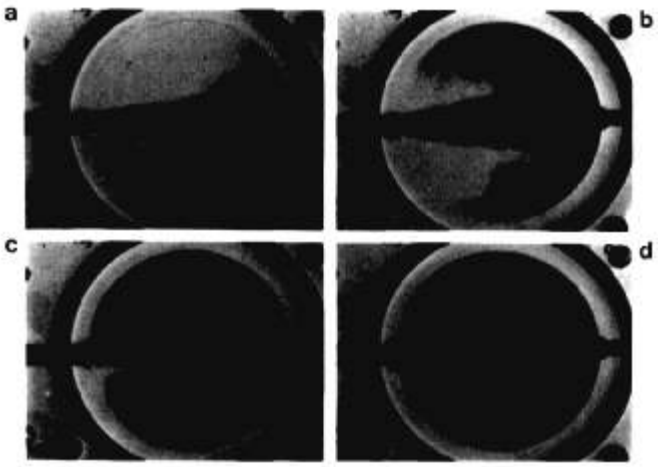


Figure 3. Pulsatile, high Reynolds number flow in FTCCC. (A) Central jet. (B) Flow impingement. (C) Evolution of the reversed flow along the curved walls. (D) Formation of the recirculating eddies.

sis, and nuclear scintigraphy using ^{99m}Tc as a tracer. The models were connected (one at a time) to the perfusion system described elsewhere.[7,9] For dye visualization experiments, the model chambers were placed on an illumination stage equipped with a video camera (Dage-MTI, Michigan City, IN). The images were recorded on video tape (AG 7300, Panasonic, Secaucus, NJ) and then acquired by an image analyzer (TN 8502, Noran, Middleton, WI). For nuclear scintigraphy, the model chambers were placed on the γ camera's collimator (SIM-400, Scinticor Milwaukee, WI). First pass images were acquired and processed using a dedicated computer system.[10]

A typical evolution of high Reynolds number flow (2045) in the FTCCC is shown in the series of photographs in **Figure 3**. The flow emerged from the inlet at 47 cm/sec at a pulsation rate of 1 Hz. The flow was turbulent throughout the entire flow chamber. Despite fast dye dispersion, we could discern flow patterns in the circular cavity. These patterns included a central jet impinging flow that is forced to reverse its direction, roll-up and formation of two symmetric vortexes, and separated flow in the vicinity of the inlet port. The time scales associated with the establishment of the coherent motion of fluid were deduced from the time records of ^{99m}Tc counts in the first pass images. A sample of such tem-

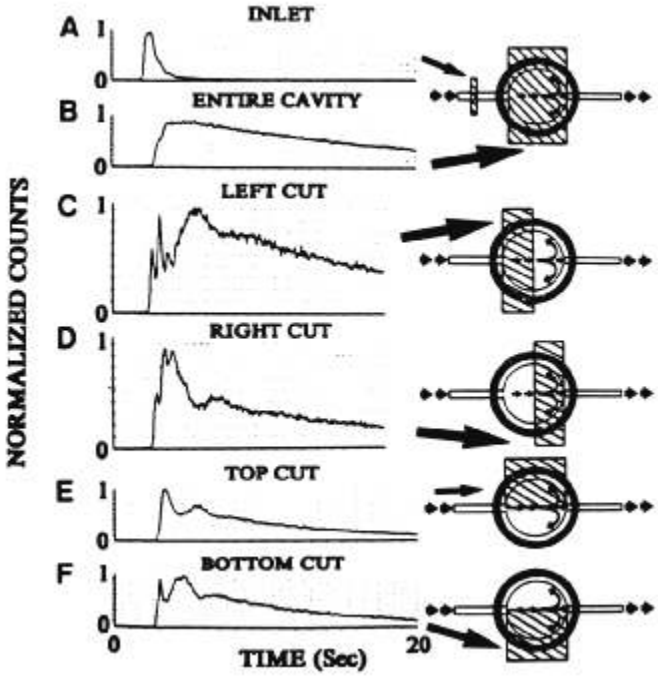


Figure 4. Temporal records of ^{99m}Tc counts in FTCCC (left-hand side) at various ROIs (right-hand side). The flow chamber was perfused by pulsating flow at a mean flow rate of 100 ml/min, and pulsation rate of 3.1 Hz.

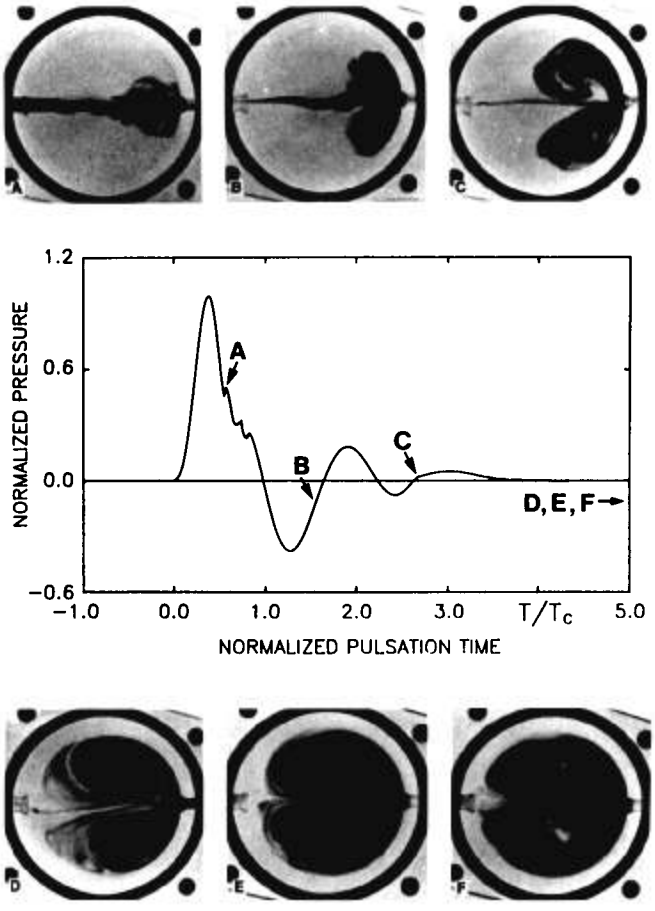
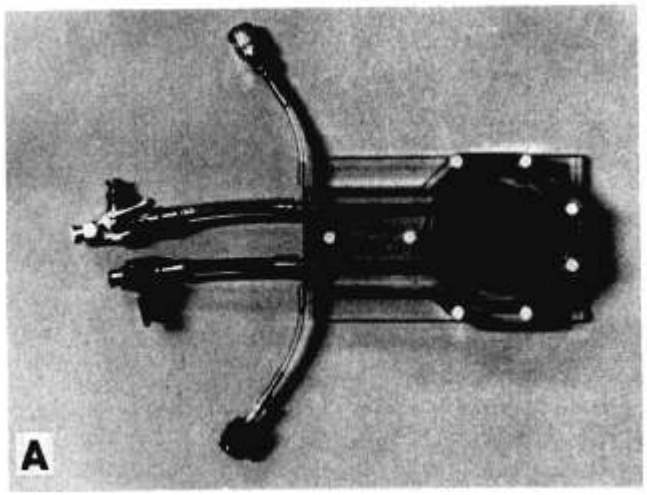


Figure 5. Pressure record and representative images of flow evolution during perfusion of the FTCCC with a single-pulse flow. The mean flow rate per pulse was 27 ml/min with a characteristic period of 0.78125 sec, and peak pressure of 29.4 mmHg.

poral signatures at various regions of interest (ROIs) is shown in **Figure 4**. In the straight conduit, the washin and washout of the bolus results in a single narrow peak, the width of which depends on the convective speed of the perfusate. In regions governed by recirculating flow, the washin related slope is sharp, but the washout is portrayed by a slowly descending curve. The various spatial oscillation modes of the recirculating eddies and the degree of their planar symmetry are traced by the numerous peaks and troughs in the ROIs. The oscillatory motion of the eddies is responsible, in part, for the formation of patches of highly disturbed flow in the vicinity of the stagnation cores, and this has an adverse effect on the endurance of the EC monolayer in these regions.

To mimic the blood flow in a beating ventricle more accurately, the FTCCC was also perfused by a single pulse flow. A characteristic signature of the pressure pulse (corresponding to a mean perfusion rate per pulse of 27 ml/min) is shown in **Figure 5**. Included in this figure is a series of images that depict the evolution of the flow inside the cavity and correlate it with the various time scales along the pressure pulse. Of particular importance was the finding that, in this type of flow, the coherence of the recirculating eddies persists long after the pressure pulse has vanished. This validates the idea that improper timing between successive pulses (pump beats) might lead to superposition of out of phase vortical flows, resulting in highly disturbed local patches of fluid that might be detrimental to blood constituents and the integrity and function of an EC monolayer.



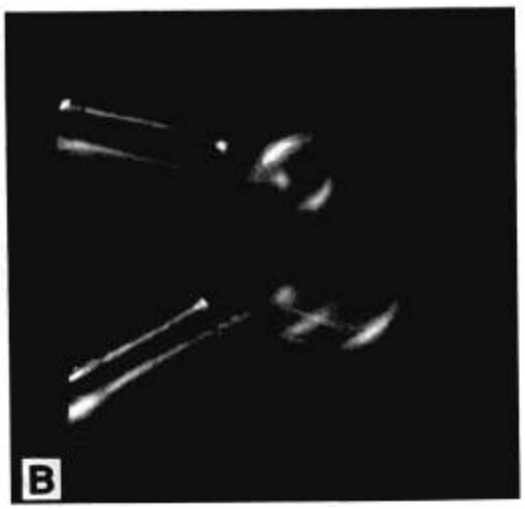


Figure 6. (A) U-type cell culture chamber. (B) Bladder of an artificial blood pump (the Milwaukee Heart).

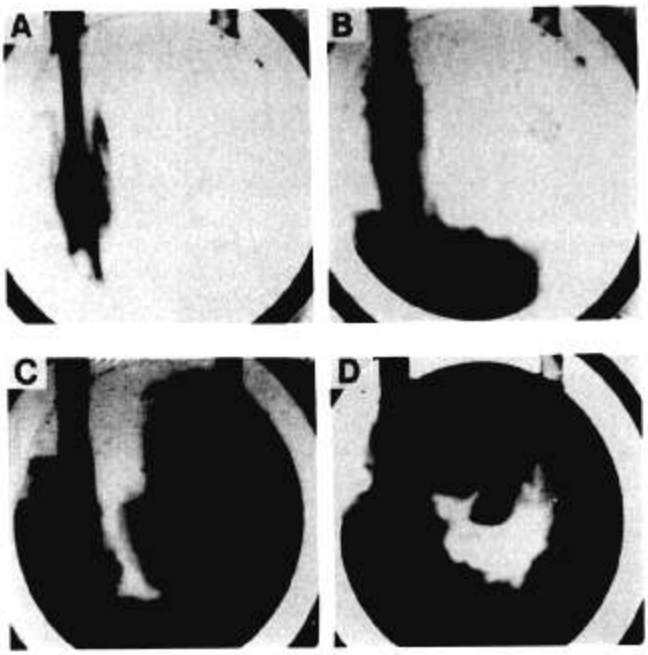


Figure 7. Pulsatile, high Reynolds number flow in the UTCCC. (A) Turbulent jet. (B) Flow impingement. (C) Evolution of the reversed flow along the curved wall. (D) Formation of the recirculating eddy.

The methods developed for studying the flow field in the FTCCC, (where the kinematics of the flow is dictated by the planar symmetry of the design), have also been implemented in studies of the flow patterns inside a U-type cell culture chamber (UTCCC, **Figure 6**) and inside the bladder of a novel artificial blood pump (The Milwaukee Heart) that was developed in our laboratory. **Figure 7** shows (in a series of photographs) a typical evolution of high Reynolds number flow in the UTCCC. In this case, the mean perfusion rate was 200 ml/min at a pulsation rate of 1 Hz. The flow emerged from the inlet port as a turbulent jet. Within a short distance, this impinged on the distal curved wall and was forced to reverse its direction. The reversed flow eventually merged with the inflowing jet, forming a central recirculating eddy and two stagnation zones, one at the core of the eddy and the other (because of flow separation), at the left hand side of the inlet port. Similar findings could be discerned from the

spatial distribution of ^{99m}Tc counts in the first pass images **(Figure 8)**. In addition to validating the dye visualization results, these scintigraphy data also permitted us to assess the dimensions and shape of the stagnation areas and their variations during the pulsation period.

In designing the blood sac **(Figure 6)**, our emphasis included eliminating the stagnation areas that were observed in the FTCCC and UTCCC chambers. In addition, we paid attention to the proper design and placement of the inflow and outflow tracts to ensure smooth entrance (or exit) of the blood into (or from) the ventricular cavity. The images shown in **Figure 9** attest to the effectiveness of this novel design. The radiolabeled bolus enters the ventricular blood sac undisturbed and is rapidly dispersed throughout the cavity, with no significant accumulation of the ^{99m}Tc counts in the entire viewing area that would indicate the presence of stagnation zones in the bladder.

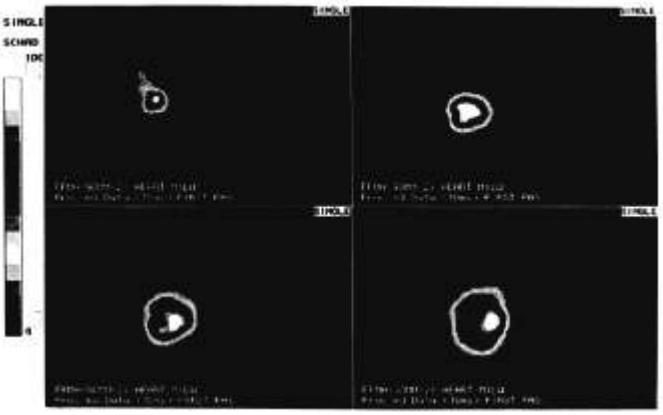


Figure 9. The first 150 msec of diastole in the Milwaukee Heart ventricle showing the wash-in of the ^{99m}Tc bolus. There is total dispersal of the bolus without any evidence of recirculating flow.

Conclusion

Successful adaptation of ECs to the dynamic environment inside a beating artificial heart is a prerequisite for their proper functioning as "nature's blood-compatible container."[11] In our study, the dynamic environment was simplified by considering only the flow induced effects. In particular, attention was focused on the possible correlation between the flow patterns and morphology and endurance of the EC monolayer in select regions in ventricle like flow chambers. The results obtained revealed distinct flow patterns in the model chambers, including flow separation and stagnation areas and recirculating eddies. For developers of endothelialized cardiac prostheses, the existence of stagnation areas in the cavity of a blood sac is a great concern. Cessation of blood flow is known to induce EC procoagulant activity.[11] This causes initial local platelet adhesion and subsequent thrombus formation. In our models, the stagnation regions were small in comparison with the total surface area of the cavity. However, even small areas are critical to maintain a functionally intact EC lining because they might involve thousands of cells.

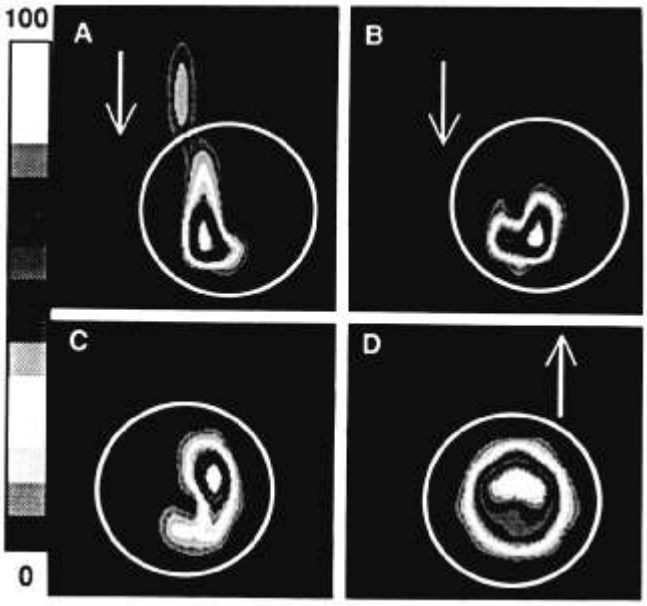


Figure 8. Evolution of high Reynolds number flow in the UTCCC delineated by the spatial distribution of ^{99m}Tc counts. (A) Turbulent jet. (B) Flow impingement. (C) Reversed flow along the curved wall. (D) Formation of the recirculating eddy.

Flow separation could be eliminated by producing a streamlined cavity design. The elimination of the stagnation core in the recirculating flow requires a periodic pumping motion of the cavity wall. This principle was used in these prostheses, but the emphasis should be on avoiding crumpling of the bladder wall if the EC monolayer is to remain intact and functional for long-term clinical use.

Acknowledgments

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