Effects of Local Geometry and Fluid Dynamics on Regional Platelet Deposition on Artificial Surfaces

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An important aspect of blood-material interactions is the activation, adhesion, and subsequent aggregation of blood platelets on the artificial surface, all of which are directly affected by local fluid dynamics. The objective of this work was to directly correlate changing local fluid dynamic conditions produced by various vessel geometries, including stenosis, aneurysm, and separate contraction and expansion geometries, with quantitative in vitro measurements of regional platelet deposition. We directly measured platelet deposition as a function of axial position along four Lexan flow chambers with axisymmetric models of these geometries using \(^{31}P\)-in-labeled platelets. Platelet deposition was maximum in observed areas of flow recirculation and reattachment and minimum in locations of high shear and separation. For the stenosis geometry, two distinct regions of increased platelet deposition were apparent, one proximal to and one distal to the stenosis throat. An approximately linear increase in platelet densities was produced in the aneurysm region, increasing in the direction of flow. Through a comparison of platelet deposition with local fluid streamline orientation, we have shown that platelet deposition is increased in certain areas due to the enhanced convective transport of platelets and blood cells to the vessel wall along locally curved streamlines with velocity components perpendicular to the vessel wall. (Arterioscler Thromb. 1993;13:1806-1813.)

KEY WORDS • fluid dynamics • regional platelet deposition • blood-materials interaction • stenosis • aneurysm • flow streamlines • \(^{31}P\)-in platelet labeling

Thromboembolic complications still constitute a significant drawback in the use of synthetic biomaterials in cardiovascular devices such as artificial heart valves,\(^{1,2}\) vascular grafts,\(^{3}\) and ventricular-assist devices.\(^{4}\) It has long been known that the process of thrombosis may be affected by a series of rheological and fluid dynamic parameters, including high rates of shear, areas of flow stagnation or recirculation, and turbulence.\(^{5,6}\) Stein and Sabbah\(^ {6}\) correlate turbulence with thrombus formation on artificial surfaces, and Yoganathan et al\(^ {7}\) report thrombus formation and tissue overgrowth on explanted Bjork-Shiley heart valves in areas of low shear and stagnation in the minor outflow region of the valve.

An important aspect of blood-material interactions is the activation, adhesion, and subsequent aggregation of blood platelets on the artificial surface. The genesis of thrombus formation is a cascade phenomenon of biochemical events induced by several clotting factors, ultimately resulting in the conversion of the soluble plasma protein fibrinogen to insoluble fibrin. The fibrin threads that are formed enmesh the aggregated platelets and clotting factors to form the thrombus, part of which may dislodge into the vasculature, inducing ischemic organ damage by the blockade of an adequate blood supply. Therefore, there has been much effort in the last two decades to study the effects of fluid dynamics on platelet kinetics.\(^{8-12}\) This work has included analytic models of convective-diffusive transport to explain the migration of red blood cells away from the wall and increased concentration of platelets near the wall of tube flow. To date, the vast majority of experimental observations includes flow visualization using latex beads; concentration profiles using freeze-capture methods; or bulk flow-type effects of high rates of fluid shear, areas of recirculation, and flow separation and reattachment on platelet aggregation and adhesion in flow chamber geometries that deviate greatly from those found in the circulation. The only direct correlations of local fluid-dynamic events with platelet adhesion and aggregation on surfaces\(^{13}\) were obtained at extremely low Reynolds numbers to simulate flow in the microcirculation.

Schmid-Schoenbein et al\(^ {13}\) summarize these observations and the local fluid dynamic phenomena leading to the thrombotic process by using flow through a stenosis as a model. In this model, local increases in shear in the throat region cause damage to and activation of platelets. Immediately downstream from the stenosis, flow separating from the throat creates a region of slowly recirculating flow conducive to aggregation of platelets.
and platelet-activating factors, and further downstream the main flow reattaches to the tube wall, creating a point of primary adhesion and subsequent aggregation of the activated platelets. In this study, we directly tested this theory by measuring regional platelet deposition as a function of axial position along a model axisymmetric stenosis using $^{111}$In-labeled platelets. We also measured platelet deposition along an aneurysm geometry and a flow chamber containing separate contraction and expansion geometries. All measurements were obtained at flow rates ranging from 0.5 to 6.0 L/min to secure the full range of flow phenomena that implanted cardiovascular devices would create, from low shear to fully turbulent flow. The overall objective of the work was to directly correlate changing local fluid-dynamic conditions produced by these various vessel geometries and flows with quantitative measurements of regional platelet deposition.

**Methods**

Regional platelet deposition was determined as a function of axial distance along the four Lexan flow chambers shown in Fig 1. A streamlined axisymmetric stenosis geometry with the form shown was chosen so comparisons could be made with previous experimental and numerical flow studies. The streamlined contractions and expansions in the flow geometry follow a sinusoidal function of the form

$$r(z) = 0.47 \pm 0.14 \left[ 1 + \cos \left( \frac{\pi z}{1.9} \right) \right]$$

where $r(z)$ is the local radius of the tube and $z$ is measured in centimeters in the axial direction of flow from the beginning of the contraction or expansion region of the tube. The plus sign is used in the expansion region and the minus sign in the contraction region. A similarly streamlined axisymmetric aneurysm geometry served as a model of recirculating flow without the upstream increases in shear stress, and the contraction/expansion geometry provided a separation of the combined effects of a gradual contraction and expansion that form the stenosis geometry. This resulted in a reduction in area (or percent stenosis) for the stenosis model of 84% and an increase in area for the aneurysm model of 156%. The straight-pipe flow chamber served as a control and allowed the separation of all other effects on the platelet deposition from those determined by local geometry and flow conditions.

For each experiment, 100 mL blood was obtained from the jugular vein of one of two conditioned female beagle dogs (10 to 12 kg) with two syringes (50 mL each), each preloaded with 10 mL acid-citrate-dextrose (ACD) solution in saline for anticoagulation. The platelets were labeled with $^{111}$In tropolone by using procedures described in detail by Dewanjee et al. and are only briefly outlined here. Platelet-rich plasma was separated from blood by centrifugation for 10 minutes at 180g. The platelet pellet was obtained by centrifugation of the platelet-rich plasma at 1000g for 10 minutes. Next, 250 to 300 $\mu$Ci $^{111}$In tropolone was added to the platelet pellet, which was suspended in 2 mL ACD-saline. After incubation at room temperature for 30 minutes, unbound $^{111}$In was removed by washing with 2 mL ACD-saline (1000g for 10 minutes). In this way, an efficiency of platelet labeling of 90% was routinely achieved.

The blood was then warmed to 37°C in an incubator (model 81, Precision Scientific) and introduced into the flow loop, taking special care to purge the system of any air bubbles, and was run at a constant flow rate for 30 minutes. Table 1 contains the flow rates and corresponding Reynolds numbers and wall shear rates (based on Poiseuille flow) used in this study. The flow loop consisted of the particular flow chamber, a water bath (model 4061, Buchi) with the temperature adjusted to maintain blood temperature at 37°C, a steady-flow peristaltic pump (model 7592, Masterflex), and approximately 2 ft of silicone rubber tubing (Fig 2). After each run the blood was drained from the flow loop and stored in the water bath for use in later runs. The flow chamber was removed from the flow loop, drained in the direction

<table>
<thead>
<tr>
<th>Flow Rate, L/min</th>
<th>Upstream Reynolds Number</th>
<th>Upstream Wall Shear Rate, s$^{-1}$</th>
<th>Stenosis Throat Wall Shear Rate, s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>300</td>
<td>84</td>
<td>1312</td>
</tr>
<tr>
<td>1.5</td>
<td>900</td>
<td>252</td>
<td>3926</td>
</tr>
<tr>
<td>3.0</td>
<td>1800</td>
<td>504</td>
<td>7872</td>
</tr>
<tr>
<td>6.0</td>
<td>3600</td>
<td>1008</td>
<td>15744</td>
</tr>
</tbody>
</table>

The diagrams showing geometries of the flow chambers machined from Lexan to simulate the various flow phenomena. a, Stenosis geometry; b, aneurysm geometry; c, contraction/expansion geometry; and d, circular cylinder geometry (control). These geometries resulted in a reduction in area (or percent stenosis) for the stenosis model of 156%. The straight-pipe flow chamber served as a control and allowed the separation of all other effects on the platelet deposition from those determined by local geometry and flow conditions.
of flow for approximately 8 minutes, and gently washed with 50 mL saline until no visible traces of blood were observed on the interior of the flow chamber. The chamber was then disassembled (into two halves) for wiping and measurement of radioactivity. The interior surface of each chamber half was wiped along predetermined lengths of sections (1 cm for the constant radius sections, 0.5 cm for the variable radius sections) with two saline-dampened cotton swabs per section. The two swabs were placed in a test tube and their total radioactivity in ^111In counts per minute was measured in an automatic gamma-well counter (model 5003, Cobra II, Packard, Inc) for 1 to 2 minutes. After each run the flow chambers were cleaned with a biodetergent solution, rinsed thoroughly with distilled water, and allowed to dry.

**Quantification of Platelet Deposition**

To compute platelet densities (number of platelets per unit surface area), the amount of platelet-bound ^111In in whole blood must first be determined. The total radioactivity in the entire volume of blood was obtained by averaging the radioactivity as measured with an ionization chamber before and after each run. A 0.5-mL blood sample was centrifuged to remove plasma, and the radioactivity in the plasma was used to determine the percentage of free ^111In. By using that, the percentage of radioactivity bound to platelets was determined, and the platelet-bound ^111In cpm per milliliter was determined by

\[
\frac{\text{Platelet-Bound } ^{111}\text{In cpm}}{\text{mL Whole Blood}} = \frac{\text{Whole-Blood } ^{111}\text{In cpm} - \text{No. Platelets}}{\text{mL Whole Blood} \times (100 - \%\text{Free } ^{111}\text{In})}\%
\]

(2)

The platelet count was determined with a Coulter counter (Coulter S+, Hialeah, Fla), and, from ^111In cpm/mL blood and the platelet count, the number of platelets per ^111In cpm was calculated.

\[
\frac{\text{No. Platelets} \times ^{111}\text{In cpm}}{\text{mL Whole Blood}} = \frac{\text{No. Platelets}}{^{111}\text{In cpm}}
\]

(3)

These methods and equations have been successfully applied previously.  

**Flow Visualization**

In addition to the platelet deposition measurements, qualitative flow visualization was performed under each experimental condition by using 400- to 450-μm-diameter neutrally buoyant resin beads (Amberlite IRA-904, Rohm and Haas, Philadelphia, Pa) suspended in a solution of 36% glycerine in saline (to match the density and viscosity of blood) and illuminated with a sheet of light that was produced by passing a laser beam (8 mW, model 1105P, Uniphase He-Ne) through a glass rod to visualize the axisymmetric flow in a single plane.

**Results**

**Verification of Methods**

The effectiveness of the cotton swab wipe method in obtaining accurate and reliable estimates of the platelet deposition on the flow chamber walls was verified in the following manner. Typical platelet densities measured from successive individual cotton swab wipes (Fig 3) indicate that the number of platelets obtained from the third swab wipe was generally 2% to 3% of the first and second swab wipes combined. However, some platelets may be lost during the wiping procedure. To estimate the number of platelets lost, it was necessary to obtain local radioactivity levels from sections without wiping. These measurements were obtained in a geometry similar to the stenosis geometry (so there would be a variation in platelet density along the axial direction of the tube) using a 10-in section of silicone tubing made
Fig 4. Diagram showing geometry configuration for a model stenosis, made by placing a collar over silicone tube, used for validation of the methods. Platelet densities were measured at each of the locations numbered 1 through 6. At each location platelet densities were estimated from cotton swab radioactivity subsequent to double wiping of region, and actual platelet densities were determined from radioactivity measurements of direct insertion of measurement section in the gamma-well counter.

Regional Platelet Density Measurement
Mean Platelet Density Over the Entire Flow Chamber

**Effects of Geometry on Platelet Deposition**

The effects of the local geometric configuration and the resulting dynamics of the flow on the variation of NPD is evident in Fig 6. The NPD curves shown are best-fit lines using data collected from five separate measurements obtained at 1.5 L/min. No significant variation in platelet density occurred in the control (straight tube) chamber (Fig 6a), where the geometry (radius) was uniform, and thus the local velocity profile remained relatively undisturbed along the length of the flow chamber. In general, platelet deposition was maximum just distal to the stenosis (Fig 6b), on the downstream side of the aneurysm cavity (Fig 6c), and in the expansion region (Fig 6d), corresponding to observed areas of flow recirculation and reattachment. NPD was at a minimum in locations of high shear and separation, ie, the throat of the stenosis, the leading edge of the aneurysm, and downstream from the contraction.

A sharp downward spike in relative platelet density occurred at the throat of the stenosis, where both high shear stresses and flow separation occurred. The NPD gradually increased toward the throat in the direction of delineate the relative effects of local geometry and flow conditions on platelet deposition on the wall, the results for the Lexan flow chambers are shown, with platelet densities normalized by the spatially averaged platelet density in the entire flow chamber. The results, then, are given as normalized platelet density (NPD).

\[
NPD = \frac{\text{Estimated platelet density}}{\text{Actual platelet density}}
\]

**Table 2. Platelet Densities for the Silicone Rubber Stenosis**

<table>
<thead>
<tr>
<th>Position</th>
<th>Estimated Platelet Density</th>
<th>Actual Platelet Density</th>
<th>Estimated/Actual, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34025</td>
<td>36600</td>
<td>93</td>
</tr>
<tr>
<td>2</td>
<td>23493</td>
<td>27747</td>
<td>85</td>
</tr>
<tr>
<td>3</td>
<td>27322</td>
<td>34100</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>31857</td>
<td>37891</td>
<td>84</td>
</tr>
<tr>
<td>5</td>
<td>20600</td>
<td>28642</td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td>31520</td>
<td>40985</td>
<td>77</td>
</tr>
</tbody>
</table>

Platelet densities are expressed as platelets per centimeter squared. Position refers to location of measurement as shown in Fig 4. The techniques used to acquire estimated and actual platelet densities are described in "Methods."
flow, and it gradually decreased distal to the throat. This phenomenon of two distinct regions of increased NPD appeared to be flow dependent, as explained in more detail below. A slight increase in NPD was also displayed upstream from the contraction region (Fig 6d). The NPD remained low throughout the extended stenotic region, and then increased sharply in the region of expansion. A much sharper increase and decrease in NPD was observed in comparison with the stenosis. The aneurysm produced a slight decrease in NPD on the proximal side, a dramatic increase along the centerline, and a continued increase of NPD along the entire distal length of the chamber (compared with the proximal-side NPD).

Effects of Flow Rate on Platelet Deposition

The regional distribution of platelet deposition also varied along the axial direction of the stenosis flow chambers as a function of flow rate and the resulting changes in shear rates and shear stresses in the fluid. For the stenosis geometry, two distinct regions of increased platelet deposition were apparent, one proximal to and one distal to the stenosis throat (Fig 7). The distal region of increased NPD was very dependent on the state of the wake region downstream from the stenosis. For the 0.5-L/min flow rate, a laminar wake was produced with an axisymmetric region of slowly recirculating flow that extended approximately 3 diameters downstream from the stenosis. The distal region of increased NPD peaked at about 2 diameters and extended to about 4 diameters from the throat, somewhat past the reattachment point of the recirculation region. At 1.5 L/min the jet was turbulent, so the region of slowly recirculating flow was disturbed and confined to approximately 2 diameters downstream. The distal region of increased NPD was likewise reduced in length, confined to a region just beyond the region of slowly recirculating flow. This trend continued for flow rates of 3.0 and 6.0 L/min. The wake became more disturbed, the region of slowly recirculating flow was reduced, and the region of increased NPD was likewise reduced. The proximal region of elevated NPD increased in magnitude with flow rate relative to the decreasing number of platelets in the distal region.

The aneurysm geometry produced less variation in NPD with flow rate than the stenosis geometry (Fig 8). In general, an approximately linear increase in NPD was produced in the aneurysm region, increasing in the direction of flow. For the 0.5-L/min flow rate, a very slow moving laminar recirculation zone was confined to the aneurysm, with the dividing streamline parallel to the upstream chamber wall. At 1.5 and 3.0 L/min, the flow in the annular region of the aneurysm was more disturbed, and the dividing streamline bulged into the main flow, causing disturbances downstream from the aneurysm. This may be causative of the slight increase in the aneurysm region of elevated NPD.
Discussion

NPD remained constant along the straight-tube flow model, but for geometries that produce flows that deviate from Poiseuille-type flow, marked variations in NPD with local changes in geometry and flow conditions were observed. As indicated in theory by Schmid-Schoenbein et al., NPD was elevated in areas of flow recirculation and reattachment. Furthermore, the extent of elevated NPD was directly correlated with the length of the recirculation zone as it varied with flow rate for the stenosis geometry.

These studies were performed in vitro by using canine blood as a model for human blood. Dewanjee et al., who compared dog, pig, and human blood, observed that dog platelets were five times more thrombogenic than porcine or human platelets for flow through a hemodialyzer. However, this property of dog blood served our experiments well due to the nature of the methods and the low material-surface contact area with blood in the flow chambers. The method of using labeled platelets to quantify NPD did not allow differentiation between adhered single platelets and platelet aggregates within the cumulative deposit, but it did provide an accurate and reliable measure of the total number of adhered platelets. Methods such as electron microscopy allow visualization of platelet deposits, but it may not be reliable in situations with large deposits due to the buildup of several layers of platelet aggregates. Flow rates were chosen to cover the full range of physiological flow that would be caused by a variety of cardiovascular devices. Steady flow through axisymmetric geometries was modeled to reduce the complexities of the problems that would arise with unsteady flow and nonsymmetric geometry (as would be the case in vivo) thus allowing a more fundamental understanding of the underlying mechanisms involved. Previous studies with stenotic geometries using vascular substrates have indicated increases in platelet deposition in the throat of the stenosis, contrary to the data presented here. This is not only most likely due to differences in adhesive forces between platelets and Lexan compared with those of platelets and the vascular wall but is also likely affected to some extent by differences in geometry and flow conditions as well as elasticity of the wall. For this study, the Lexan chambers were machined with a uniform and consistent surface and geometry that provided an appropriate environment with which to visualize the details of the flow and to correlate local flow dynamics with platelet deposition on thrombogenic artificial surfaces.

The distribution of NPD for our stenosis geometry compared favorably with results obtained by Karino and Goldsmith, who used low-Reynolds-number flow through an abrupt expansion. They measured elevated NPD in the recirculation zone immediately downstream from the expansion and reported a sharp decrease in NPD at the point of reattachment followed by a slight increase and slow decay thereafter until the fully developed value of NPD was obtained. Our measurements were unable to capture this sharp decrease at the reattachment point. This could be attributed to the relative sensitivities of each method to axial location. Whereas our method provided average NPD over a 5-mm length, they obtained NPD at 1-mm intervals, and the length of decrease in NPD at the reattachment point was on the order of 2 to 4 mm. However, as the Reynolds number was increased (>100) in their experiments, with convective transport becoming more dominant and the vortex being stretched in the direction of flow, the region of decreased levels of NPD at the reattachment point was less sharp (more spread out), and the magnitude of decrease was attenuated. The stretching of the vortex causes the angle made by the dividing streamline with the wall to become acute, as was the case in our study. Therefore, this local decrease at the point of reattachment may be dampened by decreasing the angle that the dividing streamline makes with the wall.

Karino and Goldsmith attribute this observed increase in NPD to the geometry of the local streamlines. In their view, NPD is increased in these areas due to the enhanced convective transport of platelets and blood cells to the vessel wall along the locally curved streamlines. Streamlines with components perpendicular to
the wall will cause the flowing platelets to collide with the wall at higher rates than streamlines parallel to the wall. This explanation applies directly to the recirculation region downstream from the stenosis and in the expansion region of the contraction/expansion chamber (see Fig 9a). Here we see that platelets carried by streamlines 1 through 3 all come within the critical distance for collision with the wall. The number of platelets carried within the adhesion region of the wall increases from points A through C. From point C through point D, the collision frequency should be similar, and this would be the location for maximum deposition of platelets. As the Reynolds number increases and the recirculation region decreases, the region from points C through point D shrinks in length, resulting in an NPD distribution with a stronger peak at the center of the vortex (see Fig 7).

Furthermore, this may also explain the increases in NPD observed in the entrance to the throat of the stenosis, the entrance to the contraction, and the distal side of the aneurysm. As flow enters a contraction, the flow is convectively accelerated, resulting in a contraction of the streamlines in direct proportion to the geometric contraction (see Fig 9b). We see here again, as we proceed from point A through point C, that the number of streamlines carrying platelets within the critical collision distance increases and then remains constant from this region through point D. This would lead to the prediction that NPD should increase from A through C and remain constant from C through D, as in the recirculation region. What we observed (Figs 6 and 7), however, is that NPD was elevated only in the entrance region (points A through C), followed by a dramatic decrease at point D. This phenomenon confirms the theoretical predictions of Basmadjian and others that increased platelet deposition on an artificial surface is dependent on the ability of the local geometry to enhance transport of platelets to the vessel wall as well as a diminished capacity to embolize these deposits through decreased wall shear stresses. In this particular case, wall shear stress will increase from point A through point D. It can be deduced from the NPD distributions (Figs 6 and 7) that at some point between C and D, the wall shear stress increases past the magnitude required to embolize the platelets that might otherwise have been deposited. From Fig 7, we see that as the Reynolds number increased, there was a relative increase in NPD in the throat entrance and an upstream movement of the peak NPD. This follows intuitively from the fact that as the Reynolds number grows there will be more contraction of the streamlines (resulting in more platelet collisions with the wall), and as the flow becomes turbulent the velocity profile will flatten and cause a higher increase in wall shear stress (and thus an increase in embolizing force) in the entrance region than at lower flow rates.

Folie and McIntire also showed increased platelet aggregation in regions between adjacent mural thrombi (creating flow past a cavity similar to flow through an aneurysm). We have shown here that the distribution of platelet deposition is not necessarily uniform across the aneurysm, but is again dependent on the dynamics of the flow within the aneurysm, as was the case downstream from the stenosis. A region of recirculation similar to that observed downstream from the stenosis existed in the region of the aneurysm (see Fig 9c). In this case, the vortex was skewed toward the downstream edge of the aneurysm, causing a contraction of the vortex streamlines on the distal edge of the aneurysm and an expansion of the streamlines on the proximal edge. As the flow rotated counterclockwise from point C through point A, the number of streamlines carrying platelets within the critical adhesion distance from the wall decreased. This implies that the number of platelets colliding with the wall decreased from point C through point A in the direction of the local flow in the recirculation region, or, from a different frame of reference, increased along the wall of the aneurysm in the direction of the bulk flow. For flow conditions of Reynolds numbers 300, 900, and 1800, vortex regions similar to that shown in Fig 9c were observed, thus creating the approximately linear increases in NPD in the aneurysm in the direction of the bulk flow (Fig 8). At 6 L/min, the turbulence intensities were effective in disturbing the flow within the aneurysm to the point of dominating the laminar convection mechanisms described above.

Relevance of Platelet Deposition to the Configuration of Cardiovascular Devices

Thromboembolism and anticoagulant-related hemorrhage still represent the major causes of valve-related morbidity and mortality in patients with implanted heart valve prostheses and left ventricular-assist devic-
Flow through mechanical heart valve prostheses produces contraction and curvature of the streamlines similar in nature to those depicted in Fig 9, and a detailed analysis of the local fluid mechanics in the close vicinity of the valve may provide information as to the location and magnitude of platelet deposition on the valve structure. However, more studies such as this one are necessary to elucidate the interaction of the various fluid-mechanical and mass-transport mechanisms and their relative influence on platelet aggregation and deposition. We are currently in the process of extending the qualitative correlations between fluid dynamics and platelet deposition determined in this study with quantitative correlations by using experimental and numerical simulations of blood flow in identical geometries, in nonsymmetric geometries, and under pulsatile flow conditions. We anticipate these correlations may aid in elucidating the underlying mechanisms involved in the process of flow-induced platelet aggregation and deposition.

Acknowledgments

Supported by grants from the National Institutes of Health (HL46444 and HL47201) and the American Heart Association, Florida Affiliate (Initial Investigation 91 II/10).

References

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doi: 10.1161/01.ATV.13.12.1806

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