Postoperative Changes of the Opening Angle of Canine Autogenous Vein Graft

The opening angles of 30 canine autogenous vein grafts were measured to determine the postoperative change of residual strain in the vein graft. Canine femoral veins were grafted to femoral arteries in the end-to-end anastomosis fashion. When harvested, the vein grafts were cut into short segments and the segments were cut open radially. The opened-up configurations were taken as the zero-stress states of the vessels. Opening angle, defined as the angle between the two lines from the middle point to the tips of the inner wall, was used to describe the zero-stress states. Results show that the opening angles (mean ± SD) are 63.0 ± 30.6 deg for normal femoral veins, and −0.4 ± 4.6, 6.1 ± 19.4, 25.4 ± 20.1, and 47.8 ± 11.4 deg for vein grafts at 1 day, 1 week, 4 and 12 weeks postsurgery, respectively. The postoperative changes in opening angle reveal nonuniform transmural tissue remodeling in the vascular wall. The relations between the changes in opening angle and the changes in the morphology of the vein grafts are discussed. Intimal hyperplasia is correlated to the opening angle and is suggested to be the main factor for the postoperative increase in opening angle. The longitudinal strain in the vein graft is found to decrease postoperatively.

Introduction

There is residual stress in arteries and veins (Vaishnav and Vossoughi, 1983, 1987; Fung, 1984, 1985; Xie et al., 1991). Residual stress in a vessel affects its stress state and thus its function (Fung and Liu, 1989). The zero-stress state of a vessel can be approached by making suitable cuts that release the residual stress. Fung and Liu (Fung, 1990; Fung and Liu, 1989, 1991; Liu and Fung, 1989, 1992) showed that determination of the zero-stress state is a significant way to examine the tissue remodeling of blood vessels caused by various physical (stress, etc.), chemical, and biological changes, since all the stresses are released at this state. The zero-stress state is also the reference state for the mechanical analysis of a vessel (Chuang and Fung, 1986; Fung, 1990). Recently studies have shown the changes of the residual strain in arteries as they are remodeled in hypertension (Liu and Fung, 1989; Matsumoto and Hayashi, 1991, 1994, 1996), diabetes (Liu and Fung, 1992), pulmonary hypoxia (Fung and Liu, 1991), and atherosclerosis (Matsumoto et al., 1995). However, reports on the opening angle of veins are limited to the data of normal veins (Xie et al., 1991; Liu, 1990; Han et al., 1994; Saini et al., 1995).

Autogenous vein graft is commonly used in arterial reconstruction. Significant histological changes, such as intimal hyperplasia and medial thickening, occur in the vein graft post-surgically (Dilley et al., 1988; Dobrin et al., 1988). Intimal hyperplasia is considered a major intrinsic factor associated with graft failure (Campbell et al., 1981). The veins are subjected to changed loads of higher arterial blood pressure and higher flow or higher inner wall shear stress. The remodeling of cells in the vein graft is different in each layer (Dilley et al., 1988). The nonuniformity of these changes through wall thickness can be easily seen in the zero-stress state. The study of the zero-stress state provides a new effective parameter to assess the tissue remodeling in the vein graft. There is no report of the zero-stress state of the vein graft so far.

The purpose of this paper is to show the nonuniform transmural tissue remodeling of the autogenous vein graft by studying its zero-stress state and to show how the zero-stress state changes postoperatively. This may provide useful information about the remodeling of the vein grafts as they adapt to the arterial pressure and flow, and may also lead to a better understanding of vascular remodeling in general.

Methods and Procedure

Surgical Manipulation. Seventeen mongrel dogs, 15–21 kg in body weight, were anesthetized with pentobarbital sodium at 25 g/kg. The dogs were laid supinely and draped for sterile surgery. Aortic blood pressure was measured with a catheter cannulating the left axillary artery. Longitudinal incisions were made over the femoral regions bilaterally. The femoral arteries and veins were exposed and dissected free. Several small dots of water-insoluble ink were marked on the vessel and the distances in between were measured with calipers. Then, the femoral veins were ligated and the segments between the ligatures (5–7 cm) were excised. The segments were rinsed with sterile heparin saline (60 units/ml) and put into Krebs solution aerated with 95% O₂ + 5% CO₂ at room temperature (~20°C). Arterial pieces (1 cm in length) were removed from the femoral arteries and put into the aerated Krebs bath to study the zero-stress state of normal femoral artery. After measuring the distances between the ink marks on the excised vein, four to five cm of the vein.
segments were grafted to the arteries upstream down in the end-to-end anastomosis fashion (two points mattress fixation plus running sutures, 8-0 nylon) and the remaining segments were prepared for the study of the zero-stress state of normal vein. The arterial blood vessels were then re-established and the distances between the ink marks on the vein were measured again. The grafts were covered with healthy muscle and the incisions were sutured (Lu et al., 1993). The surgical operation of vein grafting lasted 2–3 h. The residual strains in the segments of normal femoral arteries and veins in the bath were tested in the meantime.

The animals were returned to the vivarium, fed with lab chow and water in separate cages. They were randomly divided into four groups, raised for different postsurgical periods, three in the 1-day and 1-week groups, five in the 4-week group, and six in the 12-week group.

On scheduled dates, the animals were re-anesthetized. The vein grafts were re-exposed, marked with ink dots, excised en bloc, and put into an aerated Krebs bath to study the zero-stress states. The distances between the marks on the vein grafts were remeasured before and after the excision. The operation was done within 30 min after the vessels were excised.

The Zero-Stress State. The zero-stress state of a blood vessel is approximated by cutting the vessel into short segments and then cutting each segment radially (Fung, 1990). The specimens of normal femoral arteries and veins and the vein grafts (downstream half) were cut into several short segments (2–5 rings, each 1–2 mm in length), respectively. The rings were arranged in petri dishes filled with Krebs solution, photographed, and cut radially. They sprang open into sectors and were photographed again 15 min later (Han and Fung, 1991). The operation was done within 1 h after excision of the vessels. The specimens were kept in the aerated Krebs bath during the operation.

The zero-stress states were characterized by the opening angles (Fig. 1), defined as the angle between two lines connecting the middle point to the tips of inner wall (Han and Fung, 1991). The opening angles were measured from the photographs on a digitizer connected to an HP1000 computer. The error of angle measurement is within 1 deg and the error due to the bias of the middle point of the inner wall is no more than 5 deg, so the errors of the opening angles were estimated to be less than 5 deg. The opening angles of the 2–5 rings from each specimen (vein graft or normal vein) were averaged, and the average value was taken as the opening angle of the specimen.

Histology Observation. After the measurement of the opening angles, one of the segments from each vessel was quickly frozen in liquid nitrogen. Frozen sections were cut and stained with haemotoxylin and eosin stain to observe the geometric and histological changes in the vein grafts. The inner wall circumferential length, wall thickness, and intimal thick-ness were measured from the sections using a microscopy image processing system or directly under a microscope. The lumen diameter of the vessel at the unloaded condition (blood pressure removed) was approximated with the inner wall circumferential length divided by π.

The Axial Strain Measurement. The axial strain in the vessels (artery, vein, or vein graft) in vivo can be obtained from the segmental lengths of the vessels measured both before and after excision. The axial strain (λ) is defined as the segmental length of a vessel in vivo (Lviv) divided by the free length in vitro (L0):

$$\lambda = \frac{L_{viv}}{L_0}$$  (1)

Statistical Analysis. All values are expressed as mean ± SD. Student's t test was used to compare the data of different groups. A value of $p < 0.05$ was considered statistically significant.

Results

One dog of the 12-week group died of exsanguinating hemorrhage on the day of the grafting operation. All other vein grafts were patent at the time of harvesting. The blood pressures of the dogs were in the range of 15–18 kPa. The data from the right and left limbs were averaged for each animal and the average was used as the data for the animal. There was no significant difference between the body weights of different time groups, and the body weights showed no obvious change in 12 weeks postsurgery.

The Zero-Stress State. The zero-stress states of vein grafts are different from those of the normal femoral artery and vein. Figure 2 shows the photographs of typical configurations of the zero-stress states of normal femoral arteries, normal femoral veins, and vein grafts at 1 day, 1 week, 4 weeks, and 12 weeks postsurgery. The apparent increase in wall thickness of the vein in the 1 day following the graft as seen in Fig. 2 is due to the lack of contrast between the cut edge of the vessel and the wall immediately below it, as viewed from above. This problem did not arise in the histological sections from which the wall thickness measurements were made (see Fig. 4).

The zero-stress state was described by its opening angle. Figure 3 illustrates the postsurgical variation of the opening angle of the vein grafts. It is seen that the opening angle of vein graft changes dramatically postsurgically. Statistically, the

![Normal A Normal V](image)

![1day 1wk 4wks 12wks](image)
opening angles of vein grafts of the 1 day, 1 week, and 4 weeks are significantly smaller than that of the normal veins. The opening angle of 12 weeks vein graft is smaller than that of normal vein, but the difference is insignificant. Temporally, the opening angle of vein graft drops from normal (63.0 deg) to a minimum (−0.4 deg) in 1 day, and then increases gradually in the period of 1 to 12 weeks toward an asymptotic steady value. The increase is rapid in the period of 1 to 4 weeks and slower in the period of 4 to 12 weeks. The time course of opening angle changes can be described by the following equation:

$$\theta = \theta_0 - \alpha t e^{-\beta t} + \delta e^{-\gamma t}$$

where $t$ is the postsurgical time (day), $\theta_0$, $\alpha$, $\beta$, $\gamma$, and $\delta$ are constants, $\theta_0$ is the asymptotic steady value of the opening angle. The empirical values of $\theta_0$, $\alpha$, $\beta$, $\gamma$, and $\delta$ were 55.7 deg, 64.8 deg/day$^{0.0242}$, 0.0101 day$^{-1}$, 0.0242, and 7.3 deg, respectively. The average opening angle of the normal femoral artery was −6.5 deg.

**Morphological Changes.** The histological sections were observed and measured for the vein grafts and normal vessels. Typical photographs of the histological sections are shown in Fig. 4. Intimal hyperplasia is seen in 1-week, 4- and 12-week groups. The intimal thickness of the vein graft increases postsurgically. The intimal thicknesses are illustrated in Fig. 5. Significant intimal hyperplasia is seen.

The wall thickness and inner wall circumferential lengths of the normal veins and vein grafts were obtained at the opened-up configuration. Since obvious individual diameter and wall thickness variations were observed in veins before being grafted (normal veins), all the circumferential lengths and wall thicknesses of the vein grafts were normalized with respect to the corresponding values of the veins before being grafted (normal), to eliminate the individual difference. The variation of the normalized lumen diameter and wall thickness (i.e., fractional change with respect to normal vein) are shown in Fig. 6. It is seen that both the unloaded lumen diameter and the wall thickness of the vein graft increased postsurgically. However, the change of the unloaded lumen diameter was insignificant.

**Correlation of Opening Angle With Intimal Hyperplasia.** The opening angle and intimal thickness of the vein graft increase after grafting and these changes are most likely related to each other. Figure 7 illustrates the correlation between the opening angle and the intimal thickness of the vein grafts. A good linear correlation ($R^2 = 0.993$) is seen. The data point of a normal vein is far from the regression line and has been omitted from the regression analysis since the initial reduction in opening angle has no relation with intimal hyperplasia.

**The Axial Strain in the Vein Grafts.** The axial strains in all vein grafts were approximately equal when measured immediately after being grafted, since all the lengths of the

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arterial defects were approximately equal and all the vein segments grafted were approximately equal. The mean axial strain in these veins was 1.80 immediately after being grafted. This value was significantly ($p < 0.05$) less than the axial strain in normal femoral veins in vivo.

The axial strain in the vein graft decreased postsurgically. Figure 8 illustrates the postsurgical axial strains in the vein grafts. Compared to the value measured immediately after being grafted, the axial strain in the vein grafts decreased significantly after several weeks.

The axial strains in the normal femoral arteries and veins in vivo were 1.76 ± 0.14 and 1.92 ± 0.13 (mean ± SD, $n = 14$), respectively. The difference between the arteries and veins was very significant ($p < 0.01$).

**Discussion**

The Meaning of the Change in Opening Angle. The opening angle is related to the residual strain (Han, 1991; Han and Fung, 1996). Opening angle is a direct indication of the magnitude of the residual strain in blood vessels, provided that the opened-up configurations are circular or close to circular arcs (Fung, 1990). The residual stress makes the stress and strain more uniform through the vascular wall under physiological conditions (Chung and Fung, 1986; Takamizawa and Hayashi, 1987). It affects the mechanical function of the vessel in vivo by altering the stress state under physiological conditions. The opened-up configurations of most rings of the vein grafts are close to circular arcs. This study has revealed that the opening angle of zero-stress state (thus the residual strain) in autogenous vein graft changes postsurgically.

The changes of the zero-stress state are related to the morphological changes of cellular and extracellular shape and size (Liu and Fung, 1989). The change of the opening angle of the zero-stress state is a result of nonuniform tissue remodeling of the vascular wall (Fung, 1990). The opening angle increases when the inner layers have a higher growth rate than the outer layers or when the outer layers atrophy more than the inner layers, and vice versa. The opening angle of vein graft is a simple variable to quantify the remodeling in the vein graft at a unique stress state since the mechanical changes (e.g., deformation) due to external loading are not seen in the zero-stress state. Thus the change in the zero-stress state indicates that nonuniform remodeling has taken place in the vein graft. Besides the mechanical factors, such as blood flow (shear), pressure (circumferential tension), and compliance (Dobrin et al., 1988; 1989), residual strain or residual stress may be another factor that is associated with the histology change in vein graft (Han et al., 1995). The residual stress may affect the remodeling of vein graft by varying the stress distribution in the vessel.

The opened-up configuration is taken as an approximation of the zero-stress state. This zero-stress state has been verified with the results of additional radial cuts in several reports (Liu and Fung, 1989; Fung, 1990; Han and Fung, 1991). It is widely used by many investigators, although there is a report showing that some interlayer shear stress is released when the opened
ring was cut again circumferentially (Vossoughi et al., 1993). We still use the term "zero-stress state" to name the "opened-up configuration after one radial cut" in this study.

**Histological Explanation.** Significant histological changes occur to the vein graft post surgery. After grafting, endothelial denudation occurs in vein graft due mainly to the arterial blood flow, resulting in intimal edema and direct exposure of underlying connective tissue to the blood. Mural thrombosis, inflammation, and cell death are the predominant events in the first few days. Edema, vacuolation, and necrosis occur to smooth muscle cells in the media (Dilley et al., 1988). Among these many changes, although cell depositions and edema indicate a mass increase on the inner wall, the necrosis of smooth muscle cells in the media is extensive (Brody et al., 1972). Atrophy is predominant in the inner layer and would result in decrease in the opening angle (Fung, 1990). So we suspect endothelial denudation and muscular cell necrosis in the media are the predominant histological events that initiate the reduction of opening angle, although the reduction is a result of the combination of all the observed histological changes.

The opening angle of the vein graft begins to increase in the first week after grafting. The regeneration of new cells and extracellular matrix also begin in the first week (Dilley et al., 1988). Intimal hyperplasia, indicated by the presence of intimal smooth muscle cells, begins in the second week (Dilley et al., 1988). It develops rapidly in the 2- to 4-week period after grafting, and would result in the rapid increase of opening angle in this period. The fibroblasts and collagen fibers were found increasing in the vein graft media within the first 4 weeks—medial thickening occurs in this period. Due to intimal hyperplasia, the central line of the medial layer is moved outward from the middle line of the vascular wall. Thus, medial thickening would induce a decrease in opening angle.

Later, the development of intimal hyperplasia slows down. Medial thickening and the incorporation of adventitia with surrounding connective tissue, on other hand, compensate the growth of inner layers. The increase in opening angle of the vein graft then slows down and approaches an asymptotic steady value gradually—a new balance is then reached. Briefly, the increase in wall thickness arises from both intimal hyperplasia and medial thickening.

**Relation Between Opening Angle and Morphology.** The change in opening angle is related to the nonuniform tissue remodeling of vascular wall (Fung, 1990). Intimal hyperplasia is obviously a nonuniform change to the vein graft. The increase in opening angle associated with the increase in intimal hyperplasia, as shown by the correlation in Fig. 7, is reasonable. It agrees with our discussion in the preceding section and suggests that intimal hyperplasia in the vein graft is a main event associated with the changes in opening angle.

No correlation was seen between opening angle and wall thickness. This may be partially due to the individual difference in the original wall thickness of the veins before grafting. In fact, when normalized with respect to the corresponding original values of the veins before being grafted, there was a correlation between the normalized wall thickness and the opening angle although the correlation ($R^2 = 0.791$) is not as good as that between the intimal hyperplasia and opening angle ($R^2 = 0.993$).

We have shown that there is a significant increase in the intimal thickness and the wall thickness post surgically, while the lumen diameter in the unloaded state does not change much. Intimal hyperplasia compensates for the adaptive increase of vessel lumen diameter under the arterial blood pressure which is higher than the venous pressure. In fact, the lumen has been reported to narrow post surgically by some investigators (Dilley et al., 1988; Brody et al., 1972). The consistent unloaded lumen diameter in this study corresponds to the consistent flux of blood in the vein grafts (Zhao et al., 1993).

**The Axial Strains.** The axial strain in the veins has not been well studied, although the axial strain in arteries has been measured and is well known (Bergel, 1961; McDonald, 1974; Patel and Vaishnav, 1972; Vossoughi, 1992). The axial strain in the femoral vein is shown to be larger than that in the femoral artery. Our unpublished data also indicate that axial strain is larger in the vena cava than in the accompanying abdominal aorta. This may suggest that the accompanying arteries and veins are working under different axial strain in physiological condition. The veins are more flexible than the accompanying arteries. The larger axial strain in the vein is helpful to maintain its mechanical stability.

The remodeling of the vein graft is affected by the axial stress, since the growth of tissue is closely related to the stress in the tissue (Fung, 1990). The effects of different axial stress and strain on the evolution of vein graft, e.g., the histological changes and patency rate, are questions that remain unanswered and require further study.

**Comparison to Hypertensive and Diabetic Vessels.** In comparison, the opening angles of the vein grafts in this study and the hypertensive and diabetic arteries all change rapidly at the beginning, followed by a slower process of recovery. However, the opening angle in the vein graft shows a large decrease at first, contrasting with the big increase in the other cases. Furthermore, the opening angle of vein graft reaches its minimum in 1 day post surgery, while the opening angle of hypertensive pulmonary artery reaches the maximum in 12 h and the opening angles of hypertensive (aortic constricted induced) and diabetic arteries reach the maximum in 4 days and 30 days, respectively (see Fung and Liu, 1989, 1991; Liu and Fung, 1992).

We speculate that the main reasons for the large decrease in opening angle of vein graft are endothelial denudation and the damage to the inner layers, that did not occur in hypertensive and diabetic arteries. Other physiological differences, such as the loss of vasa vumrum blood supply and the nerves in the vascular wall of the vein graft, do not occur in the hypertensive and diabetic vessels.

Some of the data sets in this study have fewer animals. The numbers of animals are at the low limit of sample size (Sachs, 1984). This is due to the high cost of the animals and the good agreement between the histology data from this study and those from a previous study of autogenous vein graft by one of the authors (Zhao, 1991). The animals in this study were operated on and examined bilaterally. The data from the left and right hindlimbs were averaged for each animal. Thus, data for each animal are more accurate, representative, and reliable than unilateral data.

The results presented in this paper demonstrate a reduction in opening angle post surgery, extending the earlier results of increasing in opening angle after treating hypertension and diabetes (Fung and Liu, 1989, 1991; Liu and Fung, 1989, 1992). The opening angle of a vessel can either increase or decrease when being remodeled under different circumstances.

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