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A Nitinol "U-Clip" versus Sutured Arteriovenous Anastomosis: Local Tissue Response and Intimal Hyperplasia Development in a Sheep Model

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WHAT THIS PAPER ADDS

Arteriovenous fistula stenosis from intimal hyperplasia imparts a significant burden of morbidity and cost upon our health care system. A new nitinol anastomotic device has shown promise at reducing complications. This study sought to evaluate arteriovenous fistulae followed up to 6 months in a sheep model. It examined both the volume of intimal hyperplasia that forms and the adjacent local cellular reaction and found a distinct advantage favouring the anastomotic clip. These results suggest that the nitinol U-Clip may have utility in reducing fistula complications in clinical practice.

Objectives: This study sought to compare the local tissue response and subsequent volume of intimal hyperplasia (IH) that develops throughout the maturation of an arteriovenous fistula created using continuous/interrupted polypropylene with that of a novel, metal-alloy, penetrating anastomotic clip device.

Materials and methods: Forty-six fistulae were created in 23 sheep under a paired design using the nitinol U-Clip ($n = 23$) in one hind limb and continuous ($n = 20$) or interrupted ($n = 3$) polypropylene suture for the other. Animals were killed at 4 ($n = 3$), 14 ($n = 3$), 28 ($n = 10$), 42 ($n = 3$), and 180 ($n = 4$) days. Histological sections were evaluated for quantitative histology and immunohistochemistry.

Results: Compared with continuous polypropylene, U-Clip specimens demonstrated less intima–media area per unit length (IMA/L), proliferating cells, and tissue necrosis at all time points (MANOVA, $F = 9.8–24.1$, all $p \leq .005$; observed power >82%). Specifically, values of IMA/L were reduced by 5% ($p = .97$), 37% ($p = .02$), 33% ($p < .01$), 9% ($p = .42$), and 14% ($p = .22$) at the time points of 4, 14, 28, 42, and 180 days respectively. Proliferating cells were reduced by 75% ($p < .01$), 72% ($p = .03$), 76% ($p = .03$), 27% ($p = .31$), and 60% ($p = .01$) and tissue necrosis by 67% ($p < .01$), 58% ($p = .02$), 40% ($p = .33$), 21% ($p = .43$), 77% ($p = .11$). In a 28-day comparison between U-Clip and interrupted polypropylene the U-Clip group demonstrated a 4% ($p = .65$) reduction in IMA/L, 74% ($p < .01$) in proliferating cells and 49% ($p < .05$) in tissue necrosis.

Conclusions: These results provide evidence of reduced local tissue necrosis, proliferating cells, and IH, favouring arteriovenous fistulae created using the U-Clip anastomotic device over conventional polypropylene suture techniques most evident over the first 4 weeks.

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INTRODUCTION

A functional dialysis access point is a prerequisite for survival in patients suffering from end-stage renal failure requiring haemodialysis therapy. The autogenous arteriovenous fistula (AVF) is the gold standard for vascular access; however, it remains prone to patency-threatening stenoses, many of which occur in close proximity to the arteriovenous anastomosis. The pathophysiology of these lesions is thought to be multifactorial and particularly associated with local anastomotic factors such as compliance mismatch, intimal injury, and suture material interaction with the blood vessel wall and surrounding tissues.[1,2]

The nitinol U-Clip (Medtronic, Minneapolis, MN, USA) is a penetrating anastomotic clip device designed to reduce the use of sutures and eliminate knot tying during the creation of a circumferentially interrupted vascular anastomosis.[3] A single non-randomized, prospective human study has demonstrated

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superior patency and maturation rates for forearm AVFs created with U-Clips compared with conventional suture.[4] However, two small randomized trials have failed to show a difference in clinical outcomes.[5,6] In this present study, a reduction in intimal hyperplasia (IH) volume after the creation of an AVF using the nitinol U-Clip compared with the conventional continuous sutured technique has been demonstrated.[7] From this research a number of questions have arisen regarding local tissue trauma and cellular mechanisms by which the U-Clip achieves this effect.

The aim of this study was to further previous research by comparing this device with a conventional continuous sutured anastomosis in AV fistulae over a longer maturation period of 6 months and evaluate the local proliferative and tissue necrosis response at a cellular level. Furthermore the study sought to compare the U-Clip with an interrupted polypropylene anastomosis to determine whether observed differences were due to the suture material itself or the anastomosis configuration.

MATERIALS AND METHODS

Animals

Two-year-old crossbred Border Leicester—Merino wethers were used with the approval of the local Animal Care and Ethics Committee. They were kept within the research facility in accordance with the research guidelines of the National Health and Medical Research Council of Australia.

Surgery

The AVF surgical model has been described previously.[7] In brief, sheep were sedated, administered general anaesthesia, and then intubated. Through hind limb groin creases a 2-cm-long longitudinal arteriotomy and corresponding venotomy was performed in the proximal superficial femoral artery and vein. This length was standardized for all anastomoses and all animals. A conventional Brescia—Cimino, side-to-side anastomosis was created using either continuous or interrupted 6—0 polypropylene (Prolene, Ethicon, Johnson & Johnson, Warren, NJ, USA) in one hind limb and interrupted U-Clips in the contralateral one.[8] Flushing with heparinized saline and haemostasis were given careful attention at the time of sling release. Patency of the fistula was confirmed with pulse and thrill at the end of the procedure. The hind limb side chosen for suture/U-Clip was alternated in consecutive animals.

The nitinol U-Clip was applied with a standard needle and suturing action as described previously.[7] It was connected to the surgical needle by a fine wire and released by grasping a trigger point with the teeth of the needle holder, at which point it resumes its circular shape holding the blood vessel walls in apposition. A retention knob at the end of the clip snugs against the vessel wall to prevent it pulling through. U-Clips are placed every 1—2 mm as one would with a conventional interrupted suture.

Time points

Twenty-three sheep were utilized to create 46 AVFs. They were killed at 4 days (3 sheep, 6 AVF), 14 days (3 sheep, 6 AVF), 28 days (10 sheep, 20 AVF), 42 days (3 sheep, 6 AVF), and 180 days (4 sheep, 8 AVF).

Specimen explantation and preparation

At harvesting, hind limb dissection was carried out to identify and excise each AVF en bloc with its associated few centimetres of feeding artery and vein. The AVF was gently flushed with 10% buffered formalin so as not to remove any thrombus or IH and then fixed in 10% neutral buffered formalin for 48 hours. High-resolution X-ray was used to confirm the presence of U-Clips and aid orientation in all specimens, which were then divided through the longitudinal midpoint of the anastomosis in a cross-sectional plane and at 1—2-mm intervals either side. Half were processed for paraffin embedding after removal of the U-Clips, the other half for polymethyl-methacrylate (PMMA) embedding. The paraffin-embedded blocks were cut to 5 μm thick on slides using a Leica RM 2165 microtome (Leica Microsystems, Germany). Sections on Superfrost slides were stained with Harris's haematoxylin and eosin (H&E) and Van Gieson for histological and morphometric analysis, and sections on Superfrost Ultra Plus slides underwent immunohistochemistry for protein expression of specific markers. The PMMA-embedded specimens were cut into 10-μm-thick sections on a Leica SP 1600 saw microtome, acid etched, and stained with basic fuschin and methylene blue. The PMMA samples allowed sectioning directly through the metallic U-Clips.

Histology and morphometric analysis

Sections from each block were examined to determine and include every sample that was representative of a cross-section through the arteriovenous anastomosis. These cross-sections were then processed to determine intima—media area per unit length and luminal circumference. Digital images of H&E and Van Gieson-stained slides were taken at 1.25, 2, 4, 10, and 20× objective magnifications on a BX51 Olympus microscope with a DP2-BSW camera and software (Olympus Corporation, Japan).

Olympus DP2-BSW software was used to manually trace a line that followed the outer border of the media and a second line to trace the lumen so as to encapsulate both the intima and media. The intima and media area were calculated together rather than attempting to trace the internal elastic lamina that separates them as the neointima and media were histologically indistinguishable. The area within the first circle was calculated (B), as was the area within the inner circle (A). The intima—media area (IMA) was then calculated as $B - A$, and divided by the luminal circumference (IMA/L) to correct for artefactual exaggeration from oblique sectioning of the specimen and also vessel dilatation, as described previously.[7] This calculated area per unit length measurement was used to precisely detect any true increase in neointimal area and discount