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Experimental analysis of tensile force of individualized stents for microvascular anastomoses

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Abstract

The aim of this project was to investigate the fundamental idea of the possibility of anastomosing small blood vessels in microvascular transplant procedures by an individualized stent known from coronary angioplasty. We investigated the influence of length, dilation and differences in fabrication of the newly developed balloon-expandable stent on the tensile force of stented anastomoses. Various gripping devices were tested and validated to investigate how the length, dilatation and differences in fabrication of the newly developed stent influence the tensile force of the stented anastomosis. Overall, 66 arteries of thiel-fixed human cadavers were investigated, divided into 11 groups. The median tensile force in sutured anastomoses was 2.96 N. The stented anastomoses with 24 mm stents and Ø 3.5 mm dilation attained approximately two-thirds of F_{max}-values compared with conventional sutured anastomoses. If the stent was less dilated or had a shorter length, the maximum tensile force of the anastomosis was lower. Recent developments with an inversely oriented stent structure are expected to achieve even higher tensile force values. Further research in stent design to reduce leakage is necessary. A reduction of stent and catheter dimension is also needed to enhance the implantation method.

Keywords: microsurgery; stented anastomoses; tensile force analysis.

Introduction

The suturing technique of anastomoses introduced by Alexis Carrel in 1902 [3] is currently the gold standard in microvascular organ or tissue transplantation procedures [1] and in head and neck microvascular reconstruction [17]. However, this well-established technique has several disadvantages. In microvascular free tissue transfer for head and neck reconstruction, complications such as transplant loss or wound healing disorders can lead to serious consequences for the patient [30]. Therefore, the development of secure techniques to join the ends of two vessels is an important area of research. Overall, more than 60 patents detailing alternative techniques for anastomosing vessels can be found in the literature. Their main focus lies in gluing and laser-assisted procedures. In addition to these procedures, cuff techniques [5, 7, 10] involving connections with soluble polyvinyl alcohol (PVA) tubes as an internal stent and plastic adhesive polyethylene-glycol [29] in combination with fibrin glue [15], stick connections with vascular prosthesis or interposition connectors [21], and microporous endovasculary steel tubes combined with artificial grafts [24] have been examined. All these experimental set-ups have been fully investigated in animal models but have never reached clinical trial. The main problems have been leakages from the anastomoses, the early occlusion of the vessel, and the necessity for additional sutures for implant fixation. These methods show several advantages, but because of limitations with regard to the safety and stability of the anastomoses the suturing technique still proves superior [13, 27, 31].

Other than these sutureless techniques, another possibility for connecting microvessels is the application of staples and clips [23, 16, 32] which can be used for both arterial and venous anastomoses with a diameter starting from 0.9 mm. Disadvantages are apparent here in the handling of small diameter vessels, and sometimes stay sutures are needed. Furthermore, an impressive traumatic anchorage, which compromises the vessel and complicates any possible revision, has become evident.

The focus of the present study is to transfer the technical advances achieved in coronary angioplasty to reconstructive microsurgical procedures for anastomosing even small blood vessels (diameter 0.5 mm and less) without the requirement of further sutures. Therefore, we have analyzed modified stents normally used in coronary angioplasty. In our set-up, the stent is used as an intraluminal connection device between two vessel ends and not for expanding a stenosis. Because of capillary sprouting into the transplanted tissue after 3–4 weeks and the consequently autonomous vascularization [4, 25, 28], the so-called late thrombosis found in coronary angioplasty cases should not present a problem.

One of the major challenges for stent-based anastomoses is their mechanical properties. The used and modified balloon-expandable percutaneous transluminal coronary angioplasty (PTCA) stents are usually engineered to prevent any

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damage from the endothelial layer by having a smooth surface and architecture. Normally, no intravasal tensile force is applied to the stent. Use of the stent as a connection device requires tensile forces, which is a major challenge in the provision of a tight, leak-proof, and reliable anastomosis. The purpose of this study was to evaluate the mechanical characteristics of stent-based anastomoses with a new tensile measuring technique and to compare it with suture-based anastomoses.

Material and methods

Instrumental set-up

In our experimental set-up, we used a standard tension testing machine (Zwicki, Zwick GmbH & Co, Ulm, Germany). We performed several preliminary tests to determine the best gripping device. We evaluated two standard and three custom-made gripping devices in our preliminary studies with a silicon phantom of a blood vessel made from the material Elastosil® M 4600 A/B (Wacker-Chemie GmbH, Munich, Germany). The material properties of compliance and Young's modulus were determined according to the material properties of a coronary artery as described in the literature [11, 19]. The most frequent problem of the gripping devices was the slippage of the phantom. Only the standard mechanism with secure mounting clamps showed no slippage, and no perforation of the phantom device in the clamped area. Therefore, we used this gripping device for our validation analysis. A representation with the basic conditions for the tension tests is shown in Figure 1.

To validate our experimental set-up for analysis with biological material, we used porcine arteries. The porcine model is well-established in experimental cardiology because of the

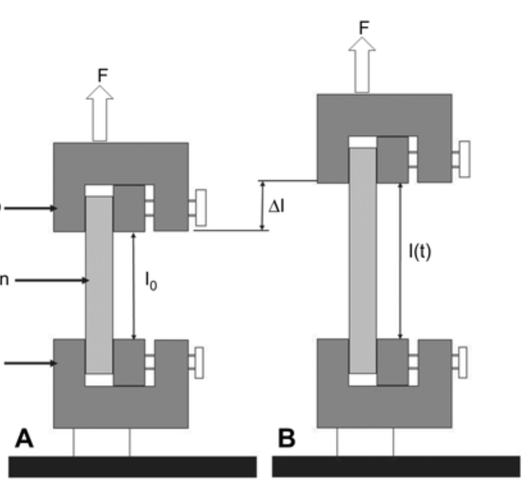

Figure 1 Representation of the standard gripping device for our experimental set-up with secure mounting clamps. A fixed clamp at the bottom, the specimen in the middle, and the mobile clamp above. (A) Apparatus in initial position with a length of 10 mm. The testing force is applied to the top clamp. (B) Specimen showing an displacement of Δl in a defined time period l(t).

high similarity in anatomy and physiology with human heart vessels [14, 20]. We dissected 18 coronary arteries from 12 pig hearts obtained from a butcher and analyzed them by using the described experimental set-up. We applied an axial force with a testing speed of 20 mm/min until the vessels ruptured. According to the clinical situation we made sure that the specimen was clamped without any preload. During surgery the vessels must be placed without a tensile force to avoid stress on the cells to ensure that there occurs no congestion in the anastomosis. The force was measured by a load cell transducer used to convert a force into an electrical signal and processed by using the software TestXpert© (Zwick GmbH & Co, Ulm, Germany). The software recorded the tension and displacement of the specimen and determined the maximum force during recording. The first maximum of tensile force of all specimens, but not the global maximum during testing, served for defining repeatable F_{max}-values as reference in our experiment. F_{max} is the force, which is the leading-in point of rupture of the (sutured) vessel or the complete slippage of the stent out of the vessels, which is terminated when the force insert the 0-N-axis. In all the preliminary tests, the porcine arteries showed no slippage. To prevent vessel rupture at the clamped area, we used polymer insoles. The experimental set-up with secure mounting clamps was used in the following experiments.

Investigation of microvascular anastomoses

We investigated 66 vessels of 17 Thiel-fixed human cadavers [26] (primarily the posterior and anterior tibial artery) and separated them into 11 groups of six vessels each. Table 1 summarizes all 11 groups. Each of the six specimens within one group was taken from a different human body. Ethical consent for human investigation was given by the Technische Universität München, Germany (2596/09).

The mean diameter of the arteries was 2.35 mm with a minimum of 2.1 mm and a maximum of 2.8 mm. In group A, the anastomoses were sutured with the single stitch technique, each with eight stitches of Ethilon® 9/0 (Ethicon, Hamburg, Germany). In all further groups we used modern expandable stents from coronary angioplasty to join the vessels ends. All the employed stents had the same open-cell design and were mounted on balloon catheters (Orbus®, Bavaria Medizin Technologie GmbH, Oberpfaffenhofen, Germany; 18 GF/2.5, 3.0, 3.5 for short stents; Orbus® 24 GF/2.5, 3.0, 3.5 for long stents). The stents were manufactured by the laser cutting of stainless steel tubes (316LVM) and exhibited an electro-polished surface. We extended our analysis to two different stent lengths, three different expansion diameters, and two modifications in fabrication from the first-tested standard stents. In groups B, C, and D, we used short stents with a length of 18 mm, dilated to an expansion diameter of Ø 2.5 mm, Ø 3.0 mm, and Ø 3.5 mm. In group E, 18 mm stents with a dilation of Ø 3.5 mm were modified with dilated ends, whereas group F, also consisting of 18 mm stents and dilated to Ø 3.5 mm, was produced without vacuum annealing. Groups G, H, I, J, and K resembled groups B–F, apart from having a different stent length, i.e., 24 mm instead of 18 mm.

Table 1 Type of anastomosis, stent characteristics, and results.

Group	Type of anastomosis	Tensile force median (mean ± SD)
A	Sutured anastomoses	2.96 (2.93 ± 0.81)
B	18 mm standard stents, dilation to Ø 2.5 mm	0.31 (0.32 ± 0.23)
C	18 mm standard stents, dilation to Ø 3.0 mm	0.21 (0.41 ± 0.53)
D	18 mm standard stents, dilation to Ø 3.5 mm	0.90 (1.10 ± 0.66)
E	18 mm stents with dilated ends, dilation to Ø 3.5 mm	1.31 (1.25 ± 0.22)
F	18 mm stents without vacuum annealing, dilation to Ø 3.5 mm	1.13 (1.21 ± 0.62)
G	24 mm standard stents, dilation to Ø 2.5 mm	0.63 (0.69 ± 0.28)
H	24 mm standard stents, dilation to Ø 3.0 mm	1.58 (1.52 ± 0.70)
I	24 mm standard stents, dilation to Ø 3.5 mm	1.77 (1.93 ± 1.07)
J	24 mm stents with dilated ends, dilation to Ø 3.5 mm	1.25 (1.27 ± 0.49)
K	24 mm stents without vacuum annealing, dilation to Ø 3.5 mm	1.59 (1.64 ± 0.85)

According to our different groups the initial length of the experimental set-up was adapted to the stent length to have the same free length of all vessels. In group A with no stent, we used an initial length (l0) of 10 mm. In groups B–E the initial length was adjusted to 28 mm, in groups G–K to 34 mm, respectively.

Statistics

Data analysis was carried out by using PASW Statistics 17.0 (SPSS Inc., Chicago, IL, USA). The median and range of tensile force were reported for descriptive purposes; for the given group sample size of $n=6$, the range corresponded to a 95% confidence interval (CI) for the median. For the simultaneous consideration of further potentially explanatory factors such as stent length, dilation, and fabrication, an analysis of covariance (ANCOVA) was employed. By means of this analysis, global tests and tests for trends were gathered followed by alpha-error-adjusted multiple comparisons via Dunnett's t-test. Covariate-adjusted means (marginal means) from the ANCOVA were reported with 95% CIs, and a two-sided p-value of <0.05 was considered to indicate statistical significance in any comparison.

Results

A representative image sequence for the tensile tests is shown in Figure 2. Starting at (A), the stent is slightly pulled out of the vessel during the appliance of the axial force (B). Beginning with a small opening (C), a split is formed (D), until the stent is visible (D). Whether the stent is pulled out of both vessel ends cannot be determined at this time (E). As expected, the stent remains in one vessel end but is also partially pulled out of the other vessel end (F). Figure 3 shows the result of tensile tests of all 11 tested groups of anastomoses. Optimal results were achieved with group A, with anastomoses sutured by the single stitch technique. The median tensile force of this group was 2.96 N with a range of 1.78–3.81 N. The corresponding differences for almost all of the tested stent anastomoses (9/10) were statistically significant at the 0.05 level by using Dunnett's t-test for multiple comparisons. Although group I showed a similar tendency, statistical significance was lacking ($p=0.066$). The stented anastomoses with 24 mm stents and a 3.5-mm dilation reached approximately two-thirds (median: 1.77 N) of the F_{max}-values of conventional sutured anastomoses. Compared with the two tested variations in production (stents varying with dilated ends and stents fabricated without vacuum annealing), the standard produced stent achieved the best results within all tested stent anastomoses with a median of 1.77 N (range: 0.85 N–3.58 N). However, in the multivariable analysis (ANCOVA), no statistically significant differences in F_{max}-values could be observed between these three groups (global test $p=0.67$; maximum difference in means: normal vs. dilated ends: 0.3 N; 95% CI -0.5 N to

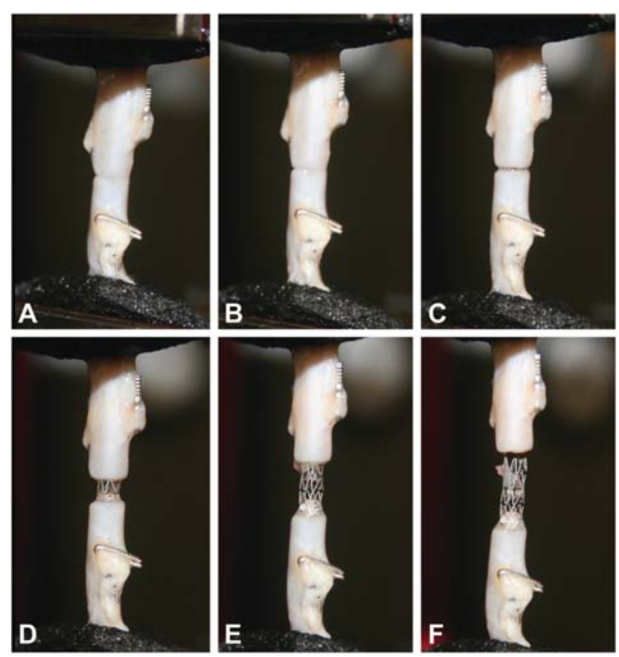

Figure 2 Image sequence of a representative tensile test (group G). Images (A)–(F) show the behavior of the stented artery during the application of the axial force. From (A) to (B), only a small movement at the artery ends can be seen. In (C), an initial dehiscence of the anastomosis is detected, which enlarges until the anastomosis breaks down (F).

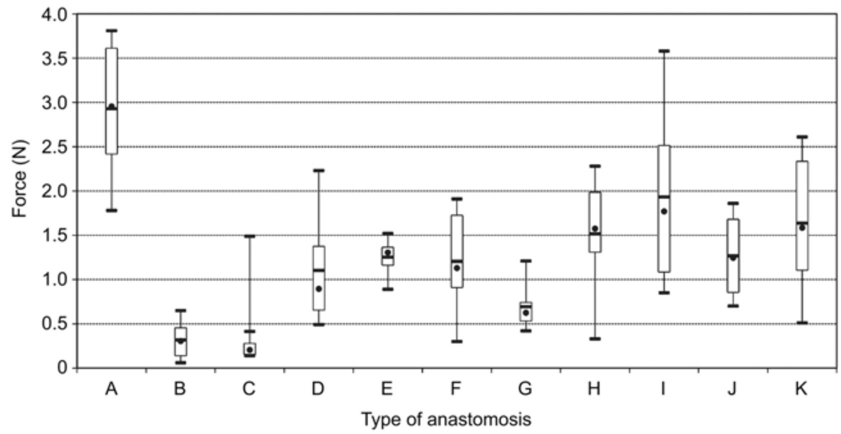

Figure 3 Result of tensile tests. The boxplots show the minimum, lower quartile, mean, median, upper quartile, and maximum for all 11 tested anastomoses. Twelve groups of different anastomosis: (A) sutured anastomosis, (B) stent length (l) of 18 mm, dilated to an expansion diameter (Ø) of 2.5 mm, (C) l=18 mm, Ø 3.0 mm, (D) l=18 mm, Ø 3.5 mm, (E) l=18 mm, Ø 3.5 mm modified with dilated ends, (F) l=18 mm, Ø 3.5 mm produced without vacuum annealing, (G) l=24 mm, Ø 2.5 mm, (H) l=24 mm, Ø 3.0 mm, (I) l=24 mm, Ø 3.5 mm, (J) l=24 mm, Ø 3.5 mm modified with dilated ends, (K) l=24 mm, Ø 3.5 mm produced without vacuum annealing.

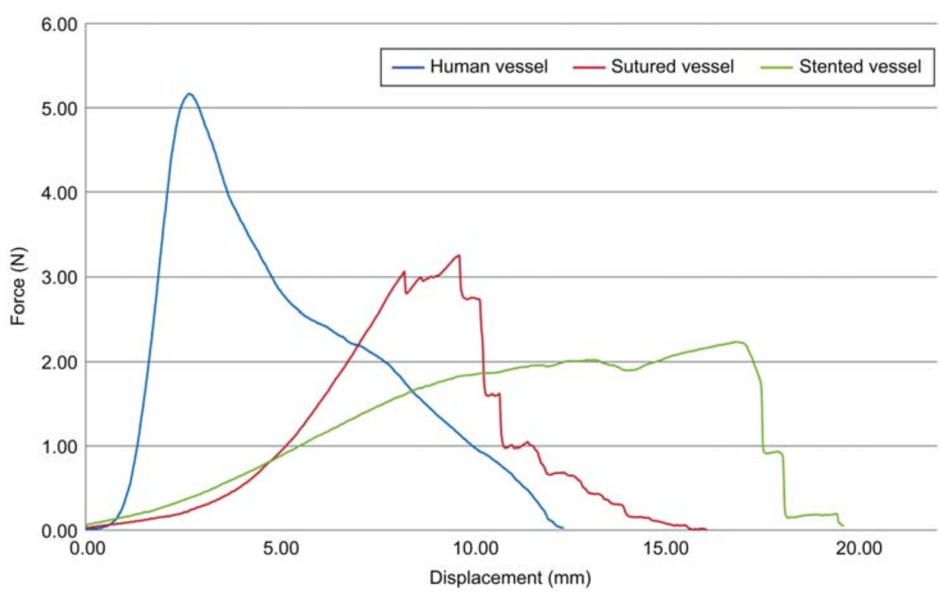

Figure 4 Development of the axial force in characteristic specimens. The human vessel shows the highest maximum values as expected. The sutured anastomosis shows lower maximum values, and the anastomosis with long stents, expanded to a diameter of Ø 3.5 mm, shows the lowest values.

+0.9 N; corrected p-value >0.99). Moreover, no significant interaction was observed between stent length and type of fabrication with regard to the effect on F_{max} levels ($p=0.37$).

Both stent length and dilatation diameters presented a significant independent association with achieved F_{max}-values (p-values marginal effects, averaged for approximately all dilations with length or averaged for all lengths with dilation: 0.001 or 0.002, respectively).

F_{max}-values were significantly elevated within the group of long 24 mm stents compared with the short 18 mm stents (mean marginal difference: 0.8 N; 95% CI: +0.3 N to +1.2 N; $p=0.001$).

Furthermore, an increase of F_{max} was observed with higher dilation levels (test for trend: $p<0.001$), whereas the most pronounced difference between the 2.5 mm and 3.0 mm dilations was statistically significant (mean difference: 1.0 N; 95% CI: +0.4 N to +1.7 N; corrected p-value=0.002). Despite an obvious tendency, the difference between the expansion diameters of 3.0 mm and 3.5 mm did not reach statistical significance (mean marginal difference 0.6 N; 95% CI: -0.1 N to +1.2 N; corrected p-value=0.13). To investigate possible differences in the effects of dilation independence on stent length, a test for interaction was performed within the linear model but demonstrated no evidence for such an effect ($p=0.38$).

Less dilated stents or shorter stent lengths exhibited only low tensile forces having a minimal value of 0.06 N for the tested anastomoses with 18 mm stent length and 2.5 mm dilation.

Figure 4 presents the curves of tension for three exemplary specimens. Two linear regions can be seen in each graph. In the first linear region an adjustment of the system appears with a minor tensile force. Finally, the second linear region shows the elasticity of the vessel or vessel-stent system. A first dehiscence in all specimens can be observed visually where the first turning point occurs in the curve (see also Figure 2B,C) and defines the clinically crucial moment of failure (leakage) of sutured and stented anastomosis.

The human vessel as the standard for artery tensile force shows the highest values, as expected. The measured forces increase rapidly to a global maximum of 5.2 N at a displacement of 2.6 mm. Subsequently, the axial force decreases rapidly with a change of slope until the vessel ruptures. This change of slope results from the unequal rupture of the vessel tissue. The group of sutured vessels, as our gold standard for anastomoses, shows a global maximum value of 3.4 N at a displacement of 9.7 mm. In addition, this group has several local maxima and value plateaus during a decrease in the force. These plateaus result from rupture of one after the other stitch. The highest maximum values of the stented anastomoses are detected in group I. The curve of the representative specimen with long stents ($l=24$ mm) and dilation to a diameter of Ø 3.5 mm shows a constant increase to a first local maximum of 2.0 N at a displacement of 13 mm. A plateau with a slight decrease of values follows this first maximum. Finally, the values increase to a global maximum of axial force with 2.23 N at 17 mm displacement, which is probably caused by another hooking of the stent into the vessel in this example, until the stent slips out of the vessel.

Discussion

The performance of microvascular reconstruction requires great care to be taken in the macroscopic and microscopic technical procedures used to maintain the blood supply of the transplant and to ensure wound healing. Occlusion of a perfusing or draining vessel caused by thrombosis in the first few weeks after the operation can lead to loss of parts or the whole transplant with consecutive postoperative wound healing complications in up to 20% of cases [8, 18, 22]. This requires further research in microvascular techniques to improve the consistency of outcomes [2].

In coronary angioplasty, non-resorbable intraluminal stents have become an integral part in the treatment of stenosis, during the past few years, even of small diameter blood vessels. However, up to 40% of patients undergoing angioplasty develop clinically significant restenosis within a year of the procedure [9]. Coronary vessels below a luminal diameter of 2.5 mm show even higher occlusion rates than larger vessel groups [12], whereas the procedural success rate, stent thrombosis, and in-hospital cardiac event rates are similar for vessel diameters over 2.5 mm. Translation of the philosophy and clinical findings from coronary angioplasty to the field of microsurgery reconstruction with free flaps should reduce late stenosis to an insignificant level when using stents in microvascular tissue transplantation because of the effect of capillary sprouting from the surrounding tissues after a few weeks [4, 25, 28]. We suggest that an increase in time efficiency and a significant drop of complication rates will occur for inexperienced surgeons as they can avoid the manual stitch technique but nevertheless ensure the vascularization of a free tissue transplant by stented anastomoses.

The goal of this ongoing research is to modify and test the mechanical characteristics of cardiovascular stents to make them suitable for stenting microvascular anastomoses during free tissue transfer. The presented preliminary tests establish that the use of a secure mounting device is a valuable method for testing the tensile force of sutured and stented microanastomoses. The combination of a gripping device with polymer insoles allows a safe testing and ensures that no specimen slippage occurs. In addition, no specimen rupture, elongation of the vessels in the clamp or failure at the clamps was observed.

The results have demonstrated that anastomoses sutured by a single stitched technique with eight stitches can hold approximately two-thirds the tensile force of a non-sutured vessel. The anastomoses with more highly dilated stents of up to Ø 3.5 mm exhibit much better gripping in comparison with other stents, whereas anastomoses with long ($l=24$ mm) and short ($l=18$ mm) stents, dilated to a diameter of Ø 2.5 mm, show unacceptably low tensile force values when challenged. Therefore, an expansion diameter larger than the vessel diameter is required for all subsequent experiments.

Furthermore, the 24 mm stents present unquestionably better results than the shorter 18 mm stents. Continuing experiments and further developments will focus on this stent length.

The cardiac stents that we have used have been marginally modified for our analyses. We first used stents (Optiray V2/18, Optiray V2/24, Optiray Medizintechnik GmbH, München, Germany) normally deployed for percutaneous transluminal angioplasty in cardiac surgery, modified first by dilation of the stent-ends, and second by fabrication without vacuum annealing. These two modifications of the PTCA stent, however, produce no advancement with regard to tensile force but caused a rougher surface, slight penetration into the intima, and a merging of the ends of the vessel.

The interconnection was induced exclusively by friction between the stent struts and the vessel. Thus, the friction of the anastomoses was not optimal and could be enhanced by an adopted stent design. However, even now, compared with the results of Colen et al. [6], which present a mean force of 96.0 N for anastomoses with eight stitches, we have reached highly satisfying results with a maximum value of 180 N for stented anastomoses (conversion: $1\ N=1\ kg\ m/s^2$; gravitational acceleration on earth: $1\ N=1\ kg\ 9.80665\ m/s^2$).

We expect further advancements in holding forces with new developments in the stent structure by inversely oriented stents. The preliminary model has shown comparable tensile forces to anastomoses sutured by the single stitch technique. Additional measurements with different set-ups have to be carried out to confirm this idea.

To make stented anastomoses suitable for modern donor-site true perforator flaps with vessel diameters of 1 mm or less, we have to reduce stent dimensions still further. Therefore, we plan to use smaller catheter diameters in combination with a newly designed crimping device.

Subsequent to satisfactory results in holding forces and in sealing and implanting methods, planned short-term and long-term animal experiments will be undertaken to investigate the patency rate and the thrombogenic influence of stented anastomoses.

Summary

We have produced satisfying results with regard to tensile force by using stented anastomoses (long 24 mm stents, dilated approximately 1 mm larger than vessel diameter). Further experimental trials should be performed utilizing a large stent diameter with an optimized stent design, a miniaturized catheter, and advanced impermeability.

Further research will include experimental modification of the mechanical, technical, and material characteristics of individualized stents and catheters to make them better suited for stenting microvascular anastomosis in free tissue transfer.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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