Annuloplasty for Valve Repair with a New Biodegradable Ring: An Experimental Study

Afksendiyos Kalangos1, Jorge Sierra1, Dominique Vala1, Mustafa Cikirikcioglu1, Beat Walpoth1, Xavier Orrit1, José Pomer2, Carlos Mestres2, Sonia Albanese3, Dhanjay Jhurry4

1University Hospital of Geneva, Department of Cardiovascular Surgery, Switzerland, 2El Clinico, Department of Cardiovascular Surgery, Barcelona, Spain, 3Bambino Gesu, Department of Pediatric Cardiac Surgery, Rome, Italy, 4University of Mauritius, Department of Chemistry, Réduit, Mauritius

Annuloplasty rings are artificial prostheses that are sutured to the native valve annulus to correct dilatation, to remodel its shape, to consolidate valve repair, and to improve coaptation between the leaflets during systole (1). Since 1968, when the first human rigid prosthetic mitral ring was implanted by Carpentier, annuloplasty technology has evolved over time from a planar, complete, rigid ring to a flexible, and then semi-rigid ring. Although currently available conventional rings respond to the needs of the adult population, no annuloplasty ring has yet been developed for surgical use in children, despite Chachques et al. (2) having proposed the use of an absorbable ring covered with a polyester sheath in pediatric patients as early as 1990. The lack of available pediatric sizes below 26 in traditional rings is due to the fact that the smallest sizes do not allow for growth of the native mitral annulus; these may therefore induce a stenotic effect in a growing child, which could worsen with time. It was mainly for this reason that the biodegradable annuloplasty ring was first designed and developed specifically for a pediatric population in 1994. Subsequently, as surgeons became convinced of the ring’s potential advantages for growth preservation of the native annulus, this was applied to adult cases during the early twenty-first century.

The present report includes primarily a description of the biodegradable ring, with its mitral and tricuspid designs. It also assesses not only the risks associated with a rapidly growing model but also some of the ring’s potential advantages, including the preservation of growth potential for the valve annulus and the avoidance of postoperative anticoagulation.

Background and aim of the study: The biodegradable ring was recently developed for mitral and tricuspid annuloplasty. The study aim was to assess the histological biocompatibility of the biodegradable ring and orifice area growth in a porcine model.

Methods: The smallest (size 16) biodegradable ring was implanted into the tricuspid annulus of 16 juvenile pigs. All animals were followed up by transthoracic echocardiography to evaluate tricuspid valve function. Animals were sacrificed at one, three, six, nine and 12 months after implantation. Macroscopic and histological analyses were performed on three sections per ring implantation site. Parameters from the study group were compared to those obtained from control animals that underwent cardiopulmonary bypass without ring implantation.

Results: Histological examination showed that the biodegradable ring was gradually replaced by fibrous tissue, with complete hydrolytic degradation within six months. The thickness of the dense fibrous tissue reached that of the initial ring at 12 months. No fibrous tissue development was observed in control animals. Echocardiography showed no signs of tricuspid valve dysfunction, a preserved ventricular contractility, and physiological growth of the tricuspid valve orifice. Macroscopic measurement of the valve orifice area confirmed that the generated fibrous tissue allows for physiological growth of the native annulus.

Conclusion: The concept of annulus remodeling using a biodegradable ring which preserves the growth potential of the native annulus opens new perspectives for valve repair procedures in the pediatric population. An undoubted contribution is also made to evolving annuloplasty technology.

Conflict of interest
Dr. Kalangos is a scientific consultant of Bioring SA, with whom he has a financial relationship in terms of royalties. The other authors do not have any conflict of interest.

Address for correspondence:
Afksendiyos Kalangos MD, PhD, DSc(Hon), FETCS, Department of Cardiovascular Surgery, University Hospital of Geneva, 24, rue Micheli-du-Crest, 1211 Geneva, Switzerland
e-mail: afksendyios.kalangos@hcuge.ch

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The biodegradable ring is implanted into the annular segment to be remodeled - as opposed to traditional rings which are implanted on the native annulus - by means of suture material extensions. These provide fixation at the anterior and posterior trigones of the mitral valve, and also fixation at the level of the posteroseptal and anteroseptal commissures of the tricuspid valve. A rapidly growing pig model was chosen to show that the fibrous tissue induced by the ring is capable of increasing its thickness in proportion to the natural growth rate of the animals. This occurs in spite of the animal’s rapid growth rate, and hence avoids the occurrence of valve stenosis with time. The rapidly growing pig model also better tests the adaptability of the ring. In the present study the biodegradable ring was implanted in the tricuspid position only for technical reasons, mainly to avoid aortic cross-clamping and problems relating to the opening of a left cardiac cavity. In addition, both histological biocompatibility and valve orifice area growth were assessed.

Materials and methods

Characteristics of the biodegradable mitral and tricuspid rings

The biodegradable ring has a curved ‘C’ segment comprised of a poly-1,4-dioxanone polymer which is colored with a blue dye (which makes it a partial ring by design as opposed to a completely circumferential one). In addition, suture material extensions equipped with a stainless steel needle are located at each extremity (Fig. 1A,B). The suture material (2/0 monofilament polypropylene) is in continuity over the entire central portion of the biodegradable ring for sizes equal to or above 26, but is interrupted in the mid portion of the biodegradable ring in pediatric sizes below 26 in order to allow for the induced fibrous tissue to grow with the native annulus over time. This ring has been designed to remodel the posterior mitral annulus or anteroposterior tricuspid annulus by inducing progressive fibrous tissue formation during its biodegradation by hydrolysis. Its specific molecular weight provides a structural memory that protects it from subsequent deformity, which is key to initiating the generation of fibrous tissue - the main cause of secondary remodeling.

Ring selection and surgical technique

The biodegradable rings are available in sizes 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36 for each of the mitral and tricuspid positions. These sizes represent the intertrigonal distances. To select the correct size, the anterior leaflet of the mitral or tricuspid valve is unfurled and the ring is chosen according to the sizer, which approximates with the anterior leaflet surface area. A size 16 tricuspid ring was selected for the present study as it corresponds to the surface of the anterior tricuspid leaflet in three-month-old pigs. The surgical technique for mitral and tricuspid ring implantation is described schematically in Figure 2A and B.

Animals and surgical technique

The experimental study was conducted in accordance with the requirements of the FDA ‘Good Laboratory Practice’ regulations (21 CFR 58 of April 1st, 2002) and the ‘Bonnes Pratiques de Laboratoire, arrêté du 14 mars 2000’ described in the ‘Journal Officiel du Ministère Français de l’Emploi et de la Solidarité du 23 mars 2000’. This study was carried out by an independent institution (Biomatech, Lyon, France) which collected, analyzed, and interpreted the data, and wrote the final report independently of the sponsor (Bioring SA). The experimental protocol was approved by the ethics committee for Biomatech.

Nineteen healthy female domestic pigs (mean age 3 months; mean body weight 39 kg; range: 30 to 43 kg) were premedicated with intramuscular ketamine (10 mg/kg) and anesthetized with intravenous sodium pentobarbital (7 mg/kg), followed by inhalation of an O2-N2O/halothane (1-4%) mixture. Muscle relaxation

Figure 1: The Kalangos® mitral ring (A) and tricuspid ring (B).
Figure 2: A) The needle attached to one of the extremities of the mitral ring is first inserted into the annulus at the level of the posterior commissure (1) and advanced as far as possible (2 mm depth), close to the insertion of the posterior leaflet on the annulus. The ring is then positioned in the annular segment by pulling up the suture material attached to it, respecting the exit axis. Subsequent insertion of the needle is made at the exit point (2) of the first stitch, respecting the principles mentioned above. The third insertion of the needle starts from the same exit point of the second insertion (3), with the needle exiting at the level of the anterior commissure. No technical difficulty was encountered in passing the needle back through the exit holes, the diameter being ca. 2 mm. Positioning of the ring in the annulus is achieved by pulling the anterior suture material up on its exit axis. This anterior suture material is then oriented by passing through the commissural annulus area, up to the anterior trigone, and is subsequently tied on itself; the posterior suture material is then oriented by passing through the commissural annulus area, up to the posterior trigone and is subsequently tied on itself. The only reason that multiple passes with the needle are required is because the specific dimension of the needle limits the length of the tissue that can be traversed with each passage of the needle. B) The needle attached to the anteroseptal part of the tricuspid ring (the short perpendicular extremity) is first inserted at the level of the posteroseptal commissure. Usually, after three insertions respecting the same technical principles as in the mitral ring implantation, the anterior part of the ring is advanced and tied at the level of the anteroseptal commissure. The posteroseptal part of the ring (the long perpendicular extremity) is then tied to the level of the posteroseptal commissure using the attached needle.
was maintained with 0.6 mg/kg intravenous curare, injected as necessary during the procedure. Intravenous cephalosporin (1 g) was administered 30 min before sternotomy in all animals. Each pig was intubated and ventilated using a respirator.

A median sternotomy was performed under sterile conditions. An aortic cannula was placed in the ascending aorta and venous drainage provided by bicaval cannulation after having controlled both venae cavae by snares. A heart-lung machine and a pediatric Minimax hollow-fiber oxygenator (Medtronic, Minneapolis, MN, USA) were used in all experiments for cardiopulmonary bypass (CPB) on a beating heart. After initiation of CPB, the snares were tightened around both caval cannulas and a right atriotomy was performed. In 16 animals, a size 16 Kalangos ring (Bioring SA, Lonay, Switzerland) was inserted into the anteroposterior segment of the tricuspid annulus via the needle at the proximal extremity of the ring, and then by pulling on the suture material extension attached to the proximal extremity of the ring, after each pass of the needle. The proximal extremity of the ring was fixed at the level of the anteroseptal commissure, and its distal extremity at the level of the posteroseptal commissure, by tying each suture material extension attached to each extremity of the ring itself. The implantation of a size 16 ring slightly reduced the tricuspid valve orifice area to that of the surface of the anterior leaflet. The right atriotomy was then closed and the animal weaned from CPB.

The remaining three animals constituted the control group, and underwent only CPB for a period equal to the mean duration of CPB of those animals in which a ring was implanted.

**Study protocol**

Transthoracic echocardiographic assessments (Dyna View, Aloka, France) were performed before surgery, at one week after surgery, and then monthly until the sixth postoperative month prior to animal sacrifice. All investigations were conducted while the animal was anesthetized with intramuscular tiletamine + zolazepam (10 mg/kg) in order to evaluate ventricular contractility and tricuspid valve function in terms of regurgitation and transvalvular gradients. Right ventricular contractility was measured by M-mode assessment, and transvalvular tricuspid gradients were measured by continuous- and pulsed-wave color Doppler. A bi-dimensional estimation of inferior vena cava diameter was performed to assess compliance of the right heart cavities.

The animals were euthanized with intravenous pentobarbital (0.1-0.3 g/kg) at either one month after surgery (four with ring implantation), at three months (three with ring implantation and one control), at six months (three with ring implantation and one control), at nine months (three with ring implantation and one control), and at 12 months (three animals with ring implantation).

A systematic gross examination of the tricuspid valve was conducted at post-mortem examination, and the valve orifice surface area measured. All three tricuspid leaflets were assessed in terms of the intensity of fibrous tissue reaction on the leaflets; their mobility was tested with nerve hooks. The ring implantation site, including the remaining tricuspid annulus, was sampled in each animal with a ring, while the entire tricuspid annulus was sampled in each of the three control animals. All samples were then fixed in 10% buffered formalin solution. Electrocardiographic controls were performed before surgery and after each postoperative month in all animals. Moreover, systemic gross examinations of organs and biopsies of spleen, liver, kidneys, lymph nodes, lungs and heart were performed. Hemato-biochemical analyses and blood cultures were performed in all animals prior to surgery and at sacrifice after one, three, six, nine and 12 months.

**Histopathological samples preparation and analysis**

After fixation, each ring implantation site was harvested with the neighboring tricuspid annulus, leaflets, and the adjacent right atrial and ventricular walls. The tissue samples were subsequently dehydrated in alcohol solutions of increasing concentration, clarified in xylene, and embedded in paraffin or in polymethyl methacrylate resin blocks. Embedded samples were then sectioned at three locations per site (anteroseptal, anterior and posteroseptal). Resin sections were obtained by using a micro-cutting and grinding technique. Resin embedding was not performed at six, nine and 12 months after implantation due to biodegradation of the ring. Paraffin sections were stained with Masson’s trichrome, and resin sections with Paragon. Histological sections were observed using a microscope fitted with ×4, ×10, ×25 and ×40 objectives. Particular attention was paid to the quality of the fibrous tissue induced by the ring.

**Results**

Overall, the ring implantation procedure was non-problematic and resulted in satisfactory positioning of the ring inside the anteroposterior segment of the tricuspid annulus. The mean CPB time was 22 ± 10 min (range: 9 to 50 min). The animals showed a normal regular growth rate during the observation period. Evolution in terms of body weight of the animals sacrificed at different time points is provided in Table I. No electrocardiographic abnormalities in favor of ischemia and/or rhythm or conduction anomalies
were detected at monthly controls after ring implantation. No endocarditis or positive blood cultures were observed in any pig at the different time points prior to surgery and sacrifice. The different organs of all implanted animals sampled at the different time points of sacrifice did not show any macroscopic abnormalities compared to those of the control group. The lung biopsies did not show any thrombotic material in the pulmonary arteries of the sacrificed pigs at the different time points. All hematological and clinical chemistry evaluations, performed in all animals at different time points before sacrifice, were within normal ranges, especially free hemoglobin levels.

**Echocardiography**

At one week and at one, two, three, four, five and six months after ring implantation, transthoracic echocardiography did not reveal any tricuspid insufficiency, or significant transvalvular gradient (peak gradients ranged from 0 to 2 mmHg) in any of the animals. On repeated echocardiographic controls, none of the animals showed any evidence of interference by fibrous tissue of the natural pliability or thickness of the neighboring leaflet tissue. Right ventricular contractility was normal (right ventricular shortening fraction 32-40%). Depression of the inferior vena cava diameter of up to 30% was measured throughout inspiration, which implied that right heart compliance was within normal ranges in all animals.

**Macroscopic observations of the tricuspid valve**

All macroscopic observations were carried out at the time of sacrifice after harvesting the heart of each animal. For all ring implantation sites, the leaflets of the tricuspid valve were normal, with no evidence of thickening, necrosis or thrombus formation. Annuloplasty ring degradation appeared to be progressive over time, being at its midpoint at one month after implantation (Fig. 3A), the two segments being attached to each other by a fibrous bundle at three months, and the ring being completely degraded and replaced by marked fibrous tissue at six months (Fig. 3B). At nine and 12

![Figure 3: Macroscopic observation of the fibrous bundle (solid arrows) at one month (A), six months (B), and at one year (C). The ring is partially degraded at its midpoint at one month, completely degraded and replaced by marked fibrous tissue at six months, with marked fibrous tissue persisting at one year after implantation. The dashed arrows show the two attachment sites of the extremities of the ring. AL: Anterior leaflet; PL: Posterior leaflet.](image)

![Figure 4: Histological analysis of tricuspid valve annulus into which the ring was implanted at one month (A) (Masson’s trichrome + Paragon; original magnification, ×10), six months (B) (Masson’s trichrome; ×40), and at one year (C) (Masson’s trichrome; ×40). Signs of surface erosion, limited amounts of material debris and inflammatory tissue were observed at one month, marked signs of implant degradation and only few signs of residual material debris were visible at six months, and a dense fibrous tissue reaching a mean diameter of 1,772 mm replaced the degraded ring at one year. AL: Anterior leaflet; RA: Right atrium; RV: Right ventricle.](image)
months (Fig. 3C), this marked fibrous tissue was macroscopically visible and a leathery consistency was palpable compared to the annulus segment without ring implantation. The fibrous tissue was found only at the annulus level, without any signs of invasion into the anterior and posterior leaflet tissue. The tricuspid valve orifice area was measured using millimetric paper; mean values in animals with ring implantation and control animals sacrificed at different time points are listed in Table II. The growth rate of the tricuspid valve orifice area was 0.25 cm² per month between 2 and 13 months after birth in animals with ring implantation, 0.26 cm² per month in the control group, and 0.026 cm² per month in healthy children during the same time period (3). In pigs, the tricuspid valve orifice area was increased by 220% between the 4th and 15th months of life (compared to that after two months of life) in both ring-implanted and control pigs; the equivalent increase in healthy children was 152%.

**Histological analysis of the tricuspid valve**

At one month, the anteroseptal, anterior and posteroseptal resin slides showed the ring to have a rounded shape and bluish staining. The ring diameter was 1600-1700 µm, and signs of surface erosion and limited amounts of material debris were observed around the ring (Fig. 4A).

At three months, in the anteroseptal and posteroseptal sections, the implanted ring was only partially degraded, with signs of superficial erosion and limited material fragmentation. The control animals showed normal histology of the tricuspid annulus. At six months, marked signs of implant degradation were observed and only a few signs of residual material debris were visible. The fibrous tissue thickened and filled the space left by the degraded implant material. This fibrous tissue was dense, and the intensity of the macrophagic and giant cell foreign body reaction decreased with respect to that of the previous time point analysis (Fig. 4B). No fibrous tissue was observed at this time in the single control animal. At nine months, marked to complete signs of implant material degradation were observed, and only a few signs of residual material particles with foamy material were still visible. The fibrous tissue of dense aspect filled the space left by the degraded implant material, whereas the control specimen showed the presence of normal fibroconnective annulus tissue. At 12 months, a small amount of foamy material was observed. The inflammatory reaction had decreased in all animals. A dense and even thicker fibrous tissue had replaced the degraded ring. The thickness of the neo-formed annular fibrous tissue was comparable to that of the initial ring thickness (Fig. 4C). The gradual progression of fibrous tissue thickness induced by the degrading ring in animals sacrificed at different time points are shown in Figure 5 (see also Table I).

<table>
<thead>
<tr>
<th>Animal age (months)</th>
<th>Postop. period (months)</th>
<th>Body weight (kg)</th>
<th>Induced fibrous tissue thickness (µm)</th>
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<tr>
<td>4</td>
<td>1</td>
<td>48 (41-54)</td>
<td>550 (535-560)</td>
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<td>6</td>
<td>3</td>
<td>67 (62-72)</td>
<td>578 (560-590)</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>96 (91-102)</td>
<td>950 (940-980)</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>142 (136-150)</td>
<td>1,250 (1,230-1,275)</td>
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<tr>
<td>15</td>
<td>12</td>
<td>195 (185-205)</td>
<td>1,772 (1,640-1,790)</td>
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</tbody>
</table>

Values are mean (range)

**Discussion**

The preservation of growth potential in the native annulus is an important issue in terms of long-term stability of valve repair procedures in children. As annuloplasty rings play a determinantal role in the success of
mitral as well as tricuspid valve repair, different attempts have been made among the pediatric population to account for growth potential, either by developing absorbable rings (2,4) or by using different biodegradable suture materials to plicate the native annulus (5-7). In 1986, Duran et al. developed an absorbable flexible ring comprised of ox bovine fibrin for temporary stenting of the tricuspid annulus in order to avoid the drawbacks of all annuloplasties and the permanent presence of prosthetic material (4). This ring, when sutured around the tricuspid annulus with a running 4-0 monofilament polypropylene, showed mild to moderate ring erosion and fibrous tissue covering the ring in dogs sacrificed between two and four weeks after implantation. However, the ring had completely disappeared by between one and two months after its implantation in nine out of 10 dogs, and in all remaining 10 dogs after two months. In 1990, Chachques et al. reported their experimental findings for the use of an absorbable ring comprised of polydioxanone covered with an extensible sewing sheath of high-porosity polyester (2). The implantation technique used was similar to that for a conventional rigid Carpentier-Edwards ring. At one year, histological analyses with this ring showed only small residual fragments of polydioxanone surrounded by collagen and elastic fibers, as well as fibroblasts with mitotic activity. No secondary valve stenosis was demonstrated in growing animals. In the present study, the most important finding was the continuation of fibrous tissue thickening, despite complete ring degradation by hydrolysis at six months. Since the fibrous reaction had not reached a plateau at 12 months, it is speculated that growth of the fibrous tissue would slow down when the animal ceased growth at 12 months, and that the space left by the degraded ring would be completely filled up. Duran et al. found the absorption time of their fibrin ring to be four to five weeks, during which time a fibrous capsule covered the ring within the first two weeks and increased in thickness thereafter (2). In none of these biodegradable rings did fibrous tissue induced by absorption of the ring adversely affect leaflet motion and pliability. In 1992, Duran et al. carried out an experimental study in sheep in order to determine the behavior of a DeVega annuloplasty performed using a 2-0 polydioxanone suture (7). Histological sections at different time points after annuloplasty showed that the polydioxanone remained practically intact during the first three months, but that the partial resorption of polydioxanone at five months allowed the tricuspid valve orifice to dilate almost to its original dimension. These authors concluded that temporary stenting of the tricuspid annulus was possible because of the gradual resorption of polydioxanone over a period of six months (7); this finding was confirmed in the present study. None of the previously described materials had the structure of the present biodegradable ring, which gradually induces a fibrous reaction during the degradation phase, the thickness of which ultimately reaches that of the initial diameter of the ring.

Total circular mitral annuloplasty performed with biodegradable suture materials (4-0 polydioxanone or 4-0 polygalactin) provided stable results at both short- and mid-term follow up, in 14 children who underwent correction of atrioventricular septal defect (5). Of these children, 77% maintained satisfactory valve competency over the long term, with a gradual increase in mitral annular size (5). However, unlike these suture materials (with which it is very difficult to precisely plicate the dilated posterior and both commissural native annulus segments according to the surface of the anterior mitral leaflet; this sometimes results in over- or undercorrection of annular dilatation), the biodegradable ring is in fact a partial ring with a specific ‘C’-curved shape, allowing for the homogeneous remodeling of the aforementioned dilated mitral annular segments using different predesigned sizes. The same technical principles of a DeVega annuloplasty are applied to the homogeneous plication of the dilated anteroposterior, as well as the posteroseptal and anteroseptal commissural tricus-

<table>
<thead>
<tr>
<th>Animal age (months)</th>
<th>Present study</th>
<th>Controls</th>
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<tr>
<td>15</td>
<td>12</td>
<td>5.5</td>
<td>220</td>
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</table>

*Relative to 2-month value.

Table II: Tricuspid valve orifice areas (TVA) in animals with ring implantation, in control animals sacrificed at different time points, and in healthy children corresponding to the time points of animal sacrifice.
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In the present study, echocardiographic investigations performed at monthly intervals showed no signs of tricuspid valve dysfunction, a preserved ventricular contractility, and physiological growth of the tricuspid valve orifice with no transvalvular gradients. Moreover, macroscopic measures of the tricuspid orifice in the sacrificed animals confirmed the preserved annulus growth potential at each observed time period, over which these animals had increased their body weights from 30-43 kg to 190-200 kg within a year. This observation proved that the suture material, when purposely interrupted in the middle of the biodegradable ring in pediatric sizes less than 26, allowed the induced fibrous tissue to grow with the native annulus over time. On the other hand, when the polypropylene suture material was kept in continuity in adult ring sizes equal to or greater than size 26, this increased the resistance of the induced fibrous tissue against the re-dilatory stretch in patients in whom the growth potential of the native annulus no longer existed. However, the polypropylene suture, when surrounded by fibrous tissue from the anterior to the posterior trigone, over the entire posterior segment and both commissural native annular segments, did not prevent the sphincter effect of the mitral orifice in systole, as shown in an ongoing clinical study where there was a 26% reduction in anteroposterior diameter in systole compared to that in diastole.

In contrast to other traditional rings that are inserted on the native annulus, implantation of the biodegradable ring into the native annulus prevents it from coming into contact with bacteria in cases of acute endocarditis. Thus, it can be used during the acute phase of infectious mitral insufficiency, as reported recently by Kazaz et al. (10).

In conclusion, the concept of annulus remodeling by inducing fibrous tissue that preserves growth potential in children undoubtedly contributes to today’s emerging advances in annuloplasty technology. The fibrous tissue, whilst apparently behaving like an autologous ring, remodels the annulus, precludes the need for anticoagulation during the postoperative period and, in theory, seems to be advantageous in cases of acute bacterial endocarditis of the mitral and tricuspid valves.

Acknowledgements

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References