Cosmetic approach to heart valve tissue engineering

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How fast are we proceeding in Tissue Engineering?

TE industry began to develop in earnest in the 1990s and grew to over 70 companies at the end of 2000.


Soon after the turn of the century the industry entered a dark period. Private sector activity decreased 20% and the capital value of publicly traded TE companies fell a staggering 90 percent.


Decrease investor interest

Failed product

Disappointing results from Food and Drug Administration (FDA) clinical trials.

HEART VALVE REGENERATIVE MEDICINE

- Single Step Heart Valve Tissue Engineering
- In vitro Biomechanical And Chemical Stimulation
- Static Seeding
- Cell Signaling
- Dynamic Seeding
- Bioreactor

- Heart Valve Tissue Engineering
- Synthetic Scaffolds
- Biologic Scaffolds
- Differentiated Cells
- Stem Cells
- Static Seeding
- Dynamic Seeding

- Immunology And Allo/Xenoantigens
- Heart Valve Tissue Guided Regeneration
- Cardio-surgical Implantation
- Allogeneic And Xenogeneic Biological Scaffolds
- Transcatheter Delivery
- Matrikine Signaling
- Decellularized, Tissue-engineered Scaffolds

Trileaflet heart valves, fabricated from novel bioabsorbable polymers (nonwoven polyglycolic-acid mesh, PGA), were sequentially seeded with autologous ovine myofibroblasts and endothelial cells. The constructs were grown for 14 days in a pulse duplicator in vitro system under gradually increasing flow and pressure conditions. The valve constructs were implanted in pulmonary position into 6 lambs for 20 weeks.

Histology of heart valve leaflet in vivo.

A - At 6 weeks, there is early organization of tissue predominantly in outer layer (magnification x50);
B - Cross section of leaflet at 16 weeks shows layered cellular fibrous tissue, which is more dense near outflow surface (top) (magnification x100);
C - Cross section of leaflet at 20 weeks demonstrates collagen (yellow), GAGs (blue), and elastin (arrow, inflow surface);

MAX IN-VIVO FOLLOW-UP: 20 WEEKS

The valve constructs were implanted in pulmonary position.

TF heart valve after 14 days of conditioning in bioreactor.

Polyglycolic acid (PGA) and poly-L-lactic acid (PLLA) mesh were adopted for fabrication of biodegradable heart valve scaffold and seeded with autologous mesenchymal stem cells for 4 weeks in *in-vitro* bottle-bioreactor.

Valves were implanted into pulmonary position of sheep and explanted after 4 and 8 months.

**MAX IN-VIVO FOLLOW-UP: 8 MONTHS**

*Figure 6.* Organization of extracellular matrix in engineered heart valve. Tissue sections of native pulmonary valve leaflet (a) and tissue-engineered valve leaflet explanted after 8 months in vivo (b) were stained with Movat pentachrome. Three tissue layers of fibrosa (f), spongiosa (s), and ventricularis (v) are enriched with specific extracellular matrix components. Areas in fibrosa and ventricularis were viewed under higher magnification (c, d). Arrows point to layer of elastin observed on ventricular side of tissue-engineered valve leaflet. Scale bar, 50 μm.

Endothelialization of tissue-engineered heart valve. Valves before implantation (a) and 8 months after implantation (b) were stained with anti–von Willebrand factor antibody. Complete and uninterrupted lining of endothelium is indicated by arrowhead. Scale bar, 50 μm.

MAX IN-VIVO FOLLOW-UP: 20 WEEKS

A, Measurements of smallest and largest conduit diameters by magnetic resonance imaging. B, Comparison of conduit diameter at time of explant with diameter measured by magnetic resonance imaging. C, Comparison of cusp length (**dark circles**) and width (**open circles**) over the study period. With the cusp at the top and the conduit wall at the bottom of the image (C, cusp; S, sinus; W, conduit wall).

A percutaneous tri-leaflet heart valve scaffold were fabricated from non-woven polyglycolic-acid mesh (PGA) covered with poly-4-hydroxybutyrate (P4HB); after seeding with primate bone marrow-derived monocluear cells the construct was loaded into the delivery system; the synthetic valve was finally implanted in a primate animal model; max in-vivo follow-up: 4 weeks.

Scanning electron microscopy of the polyglycolic acid-poly-4-hydroxybutyrate scaffold (A and B), primate (C), and human (D) control leaflets. In most areas the surface of the 4 week explants showed confluent (E and F) or initial (G) endothelial coverage. In some areas the surface remodelling was still evident involving thrombocyte attachment (H) and leucocyte attraction (I).

**SYNTHETIC SCAFFOLDS**

A percutaneous Tri-leaflets heart valve scaffold was fabricated from non-woven polyglycolic-acid mesh (PGA) covered with poly-4-hydroxybutyrate (P4HB); After seeding with ovine vascular derived cells the construct was exposed to dynamic condition for 10 days; A decellularization protocol was applied (0.25% Triton x-100 plus 0.25% DOC); The decellularized graft was reseeded with sheep/non human primate bone marrow derived mesenchymal stem cells under static condition for 3 days.

**IMPLANTED IN AN ANIMAL MODEL FOR +8 WEEKS**

In-vitro functionality of cell-populated and decellularized TEHV. (A) After decellularization the TEHV showed improved opening and closing behavior when exposed to pulmonary conditions, although some prolapse of the leaflets (indicated by black arrows) was observed. (B) Closure of the cell-populated valve leaflets was hampered by retraction of the leaflets.

staining), chondrogenic (E; toluidin blue staining), and adipogenic (C; Oil red-O staining) cell lineages. After reseeding the decellularized TEHV, Haematoxylin and Eosin (H) and DAPI staining (E and F) confirmed the attachment of cells, which formed a confluent layer after 3 days, as demonstrated by SEM (G–I). Scale bars in: A and C represent 25 μm; B represents 100 μm; D–F represent 200 μm; G–I represent 1 mm, 100 μm, and 10 μm, respectively.

A percutaneous tri-leaflet heart valve scaffold were fabricated from non-woven polyglycolic-acid mesh (PGA) covered with poly-4-hydroxybutyrate (P4HB); After seeding with ovine amniotic fluid cells the construct was loaded into the delivery system; The synthetic valve was finally implanted in the ovine fetus; Acute: 30min (n=3) Mid-term: 300 min (n=2) MAX IN-VIVO FOLLOW-UP: 1 WEEK

Histology of explanted tissues. Comparative histology of native leaflets (a–d), acute explants (e–h), mid-term explants (i–l), and long term (BRGA) explants (m–t). H&E-staining shows the decrease of originally seeded fibrous matrix with the time in vivo. Masson and eVG stainings demonstrate initial collagen formation in the long-term explants (o, s) with lack of collagen at earlier stages. Presence of α-SMA positive cells was also only detectable in the late explants stages (p). A central scaffold core was visible in all explants (red arrows; e,L,m,q; 58–250 μm). In midterm explants SEM analysis revealed initial fibrous deposition (u) combined with cells visible on the surface (v; 58–20 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A percutaneous tri-leaflet heart valve scaffold were fabricated from non-woven polyglycolic-acid mesh (PGA) covered with poly-4-hydroxybutyrate (P4HB); after seeding with ovine bone marrow mononuclear cells the construct was loaded into the delivery system; the synthetic valve was finally implanted in ovine model.

**MAX IN-VIVO FOLLOW-UP: 2 WEEKS**

*Histological Analysis of TEHV Explants*

On histology, acute explants showed clear cellular infiltrates and fibrin formation in hematoxylin and eosin staining (A to D) (magnification 5× and 10×). Interestingly, this cellularity only slightly increased in the tissues of the 24-h explants (images not shown), whereas all later explant stages at 1 week and 2 weeks showed a clearly increased cellularity (E to L) (magnification 5× and 10×). TEHV = tissue-engineered heart valve(s).
THE LAST 5 YEARS HAVE SEEN A CONSIDERABLE IMPROVEMENT OF THE TECHNIQUES:
UNFORTUNATELY THESE AMELIORATIONS ARE COUPLED WITH A REGRESSION OF THE CLINICAL APPLICATION OF SYNTHETIC HEART VALVE CONSTRUCTS.

COSMETIC TISSUE ENGINEERING

(A) Two day implant: inflammatory changes seen on the outside of the graft only.

(B) Six week implant: the conduit is completely covered with a thick fibrous sheath on the outside. There is a perforation of one leaflet cusp.

(C) One year implant: The graft is completely encapsulated with a fibrous sheath on the outside. The leaflets are virtually absent.

Photomicrographs at a low (original magnification × 100) and b high (original magnification × 200) power of the venous anastomosis of an explanted bovine ureteric arteriovenous graft stained with haematoxylin and eosin, showing transmural fibrinoid necrosis, lymphocytes, histiocytes and eosinophils. c Section of unused bovine ureter immunostained for galactose (α1 → 3) galactose (α-Gal) (original magnification × 100)

Photograph of the left shoulder of a 25-year-old man with aneurysmal dilatation of a bovine ureteric axillary–innominate arteriovenous graft 2 months after operation
THE MAIN HURDLE WITH BIOLOGIC SCAFFOLDS


Histology of decellularized a pulmonary valve allograft explanted at 52 weeks. A, Low-magnification view depicting the recellularization of the pulmonary artery and basal region of the cusp. Pannus is seen on the luminal surface of the pulmonary artery extending onto the inflow surface of the cusp. Note the presence of focal endothelialization on the outflow surface of the cusp. (Original magnification 100×, hematoxylin and eosin.) B, Higher-magnification view showing rare focal sites of recellularization of the cusp. (Original magnification 200×, Movat pentachrome stain [collagens, yellow; elastic fibers, black.])

Porcine pulmonic valves were decellularized with Triton X-100 plus ammonium hydroxide; the valves were grouped as follows: glutaraldehyde fixed, seeded with sheep endothelial progenitor cells or conjugated with anti-human CD133 monoclonal antibody; finally, the samples were implanted in sheep animal models.

**MAX IN-VIVO FOLLOW-UP: 3 MONTHS**

The valves were seeded with progenitor cells or conjugated with antibodies. After 3 months in vivo, the valves were assessed for de novo collagen formation.

**Formation and degradation of extracellular matrix (ECM) components.**

- **A.** Using Herovici stain on sections of leaflets after 3 months in vivo, all groups exhibit signs of new collagen formation (blue staining). In un-conjugated leaflets, this staining is limited solely to the leaflet edge whereas it is more widespread in the other groups. Conjugated valves clearly have the largest amount of new collagen formation at this time point.
- **B.** Remodeling requires both the production and degradation of the ECM. Here, matrix metalloproteinase-9 (MMP-9) activity is assessed using immunohistochemical techniques. Conjugated valves show widespread MMP-9 activity throughout the inner lamina of the leaflet. This activity is absent in un-conjugated leaflets.


**BIOLOGIC SCAFFOLDS**

**TEN YEARS of PADOVA TRICOL EXPERIENCE**


**Iop L et al.** The influence of heart valve leaflet matrix characteristics on the interaction between human mesenchymal stem cells and decellularized scaffolds. Biomaterials **2009;**30(25):4104-4116.


Valvular milieu and Cell colonization

Iop et al, Biomaterials 2009;30:4104-4116

Heterotypic interaction
Valvular milieu and Cell colonization

Heterotypic interaction

Iop et al, Biomaterials 2009;30:4104-4116
Valvular milieu and Cell colonization

Homotypic interaction

VS-PVLs

TEHV macroscopy before implantation (A); distal view of TRICOL TEHV from the outflow tract, 15 months from in vivo implantation (B); focus on the TRICOL allograft cusps 15 months after implantation: no macroscopic evidences of valve deterioration (C).
Iop et al., submitted

Implanted AoV

Implanted aorta

HE 160x

HE 160x
Ex vivo analysis of regenerated aortic roots in pulmonary position

Iop et al., submitted
Extracellular matrix and cell engraftment:
Conservation and Synthesis of new elements

Leaflet at 15 months from the implant

lop et al., submitted
Extracellular matrix and cell engraftment:

Stem cells

Leaflet at 15 months from the implant

Iop et al., submitted
THE PRODUCTION OF A BIOLOGIC SCAFFOLD FOR HEARTVALVE REGENERATIVE MEDICINE THROUGH THE APPLICATION OF DECELLULARIZATION PROCEDURES IS VERY DIFFICULT AND NEEDS THE COMBINATION OF MANY SKILLS: BIOLOGICAL, BIO-MOLECULAR, BIO-ENGINEERING, SURGICAL, ETC.
HOWEVER, IN COMPARISON TO THE USE OF SYNTHETIC SCAFFOLDS, THIS APPROACH IS TRANSLATED IN A GREAT TIME SAVING.

IN FACT, A GOOD BIOLOGICAL SUBSTRATE DOES NOT REQUIRE BIO-REACTOR PRECONDITIONING OR CELLULAR PRE-SEEDING.
Up to date, heart valve regenerative medicine has been able to achieve a reliable, safe and effective clinical application only for human-derived materials, being still far for synthetic or xenogeneic biological scaffolds.
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