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(54) **Title**: AN APPARATUS AND METHOD OF CULTURING TUBULAR AUTOLOGOUS TISSUE GRAFT

(57) **Abstract**: A novel apparatus and tissue graft for use in regeneration, reconstruction or repairing of urological structures and surfaces, and also a method of culturing tubular autologous tissue graft are disclosed in which urothelial cells are grown on a biocompatible scaffold which is encircled by biodegradabte and/or biocompatible shaped setting material The biocompatible scaffold is then implanted at a site where tubular structure is to be repaired or replaced. The scaffold is removed, leaving the cells inside, after 2-3 days or after the cells begin to adhere with indigenous urethral tissues.

# An Apparatus and Method of Culturing Tubular Autologous Tissue Graft

## Field of the Invention

[0001] The technical field of present invention is tissue engineering; in particular, the present invention relates to an apparatus and a method of culturing tubular autologous tissue graft for use in replacing or repairing damaged or diseased tubular structures and prevention of stricture of tubular structures.

## Background of the Invention

[0002] Tubular structures in the mammalian anatomy are prone to damage and disease commonly referred to as strictures whereby the circumference of the tubular structures is reduced by the development of fibrous scar tissue, post infection, trauma, Sexually transmitted diseases (STD) or medical intervention, more particularly catheterization.

[0003] Urethral stricture for instance is a condition that leads to obstruction in the urinary flow due to fibrous tissue formation leading to narrowing of the tube. Urethral strictures arise from various causes. Injury may be due to placement of a catheter, trauma following endoscopy or due to invasion of foreign bodies or by sexually transmitted diseases. A stricture develops as injuries heal. It may also be caused by external pressure from an enlarging tumor near the urethra.

[0004] The Urethral stricture has very significant medical as well as social impact on the patients. It is also important to have an accurate diagnosis and assessment of the location and length of the urethral stricture, and also to identify the underlying cause and thereby to overcome it.

[0005] Common methods of treatment include surgery of genitor-urinary rectal diseases or tubular structures. Surgery is normally carried out by replacing the fibrous tissue with

grafts, xenografts or heterograft using stents which are placed in urethra for the treatment.

[0006] Anthony Atala in US5851833 discusses about artificial matrices for the growth and implantation of urothelial cells on biodegradable, biocompatible, fibrous matrices formed of polymers and then implanted inside. Histological analysis demonstrates inflammatory response due to foreign body reaction when implanted *in vivo*.

[0007] Kropp *et al.* in US7122200 discloses a method of seeded tissue engineering techniques utilizing xenografts on a tissue culture frame to regenerate urinary tissue and restore normal urinary function.

[0008] Knapp Jr. *et al.* in US5762966 discusses about a method for surgical repair of a diseased or damaged bladder by replacing it with sub mucosal tissue of vertebrates. This method ends in complications like calculi formation due to the use of foreign materials.

[0009] Also known in the prior art is the usage of heterologous patch graft in repairing the stricture. The patch graft however does not solve the problem entirely since fibrous tissues are present even after the treatment. Therefore there is a need to overcome these by the use of an engineered urethral mucosa as a graft in tubular form.

[0010] The present invention discloses an apparatus and method of engineering an autologous graft in tubular form for use in treatment of tubular strictures, including urethral stricture, which overcomes the shortcomings in the prior art by eliminating graft rejection (even when used in patch form) and the need for Human Leukocyte Antigen (HLA) typing; preventing reemergence of scar tissue; and preserving biomechanical properties of the tissue.

## Objects of the Invention

[0011] It is an object of the invention to provide an apparatus and bioengineered tubular autograft for repairing or replacing damaged or diseased portion of tubular structure.

[0012] It is yet another important object of the invention to develop a safe and simple tissue graft for replacing or repairing diseased or damaged tubular structure.

[0013] It is yet another important object of the invention to repair damaged or diseased portion of tubular structure using autologous tissue graft.

[0014] It is yet another important object of the invention to avoid excessive inflammatory response by using autologous cells.

[0015] It is yet another important object of the invention to be used in prevention of strictures in tubular structures.

[0016] It is yet another important object of the invention to develop a tubular or other autologous tissue graft which ensures preservation of biomechanical properties of the tissue.

## Summary of the invention

[0017] Accordingly, to meet the stated objectives and to overcome the disadvantages of the prior art, the invention discloses a novel apparatus and autologous tubular tissue graft for use in repairing or replacing a damaged or diseased tubular structure and prevention of tubular stricture, said apparatus and tissue graft comprising a biocompatible scaffold encircled by a biocompatible and/or biodegradable shaped setting material with grown autologous cells. The biocompatible scaffold comprises a biocompatible polymer or biocompatible, non toxic metal and provided with a thread.

[0018] Also, described is a method of tissue engineering autologous cells in tubular form for use in repairing or replacing damaged or diseased tubular structure and prevention of

[0001] tubular stricture, comprising a biocompatible scaffold; growing autologous cells on the biocompatible scaffold, to thereby create a tissue structure, or segment thereof, suitable for implantation at a site where tubular structure is to be replaced or repaired.

## Brief Description of the Drawings

Fig 1.1: Structure of the male urethra

Fig 1.2: Stricture in male urethra

Fig 2.1: Excision of the Mucosa and opening of the Urethra to allow flow of urine.

Fig 2.2: Culture of Autologous Urethral Mucosal Tissue

Fig 2.3: Novel Method of Tubular Culture of the Autologous Urethral Mucosal Tissue

Fig 3.1: Placement of Tubular Graft in the affected region of the Urethra

Fig 3.2: Novel LDP Scaffold with Silk Thread

Fig 3.3: Attachment of Autologous Urethral Mucosal Tissue in a Tubular Form in the affected region

## Detailed Description

[0021] This invention relates a novel apparatus and tissue engineered graft of autologous tissue for use in repairing or replacing damaged or diseased tubular structure and prevention of tubular stricture in human body.

[0022] Damage or disease may cause obstruction or narrowing of the path in tubular structure, such as urinary, circulatory, nervous systems and the like. The damage or disease of the tubular structure can be treated by tissue engineering using autologous cells.

[0023] Disclosed is a tissue graft construct for use in repairing or replacing diseased or damaged tubular structure and use in prevention of disease in the tubular structure.

[0024] The device and tissue graft of the present invention mainly consists of a biocompatible scaffold sized in the desired dimensions coated with biodegradable and/or biocompatible membrane or shaped setting material. Autologous cells are cultured and reconstituted over the biodegradable and/or biocompatible membrane and the cells are allowed to grow on the biocompatible scaffold *in vitro*.

[0025] In accordance with this invention tissue graft constructs comprising autologous mucosal tissues have been found useful to repair or replace damaged or diseased tubular structure.

[0026] The present tissue graft derived from autologous mucosa and fibroblast cells. In one embodiment, the tissue graft is derived from autologous urothelial cells and in another embodiment derived from skin cells.

[0027] The construction of the device or auto graft in accordance with the invention is to implant at a site where tubular structure is to be repaired or replaced and for use in prevention of tubular disease in human and/or animals.

**Tissue graft construction:**

The tissue graft construction comprising following steps:

a) Isolation of autologous urethral mucosa cells

[0028] Biopsy sample of autologous urethral mucosal cells are obtained. The isolated cells are transported in a suitable transport medium, preferably, a mixture of Hank's phosphate buffer solution and antibiotics. Other transport medium known in the art may also be used. The transported sample is treated with Metalloproteinase enzymes, preferably Dispase at about 2° C to 4° C. Mucosa cells and fibroblast cells are obtained separately.

[0029] The suitable antibiotics used in the above process include Penicillin, streptomycin, and fungizone.

b) Expansion of autologous urethral mucosa cells *in vitro*

[0030] The separated mucosa cells of the previous step are cultured in 24 well plates for 2 to 3 days. Then, in 10 mm Petri dishes for 15-20 days and further expanded in 35 mm plates using suitable growth medium.

c) Expansion of autologous fibroblast tissue *in vitro*

[0031] The separated fibroblast cells are treated with digestive enzymes at a temperature in the range between 30° C to 45° C, preferably between 33° C to 41° C. Connective proteins are removed and pure fibroblast cells are obtained. The obtained fibroblast cells are expanded in fibroblast growth medium and cultured in 35 mm well plate.

[0032] Suitable digestive enzyme to be used in the above process includes chymotrypsin, trypsin and collagenase, preferably, collagenase. The process and the mentioned enzymes are known in the prior art.

d) Growing autologous cells on biocompatible scaffold *in vitro*

[0033] The cultured urethral mucosa and fibroblast cells obtained in the previous steps are combined and reconstituted on a biocompatible and/or biodegradable shaped setting material coated on biocompatible scaffold and the cultured cells encircling all around the scaffold. The cells are allowed to grow for one or two days *in vitro* using keratinocyte and fibroblast growth media.

[0034] The biocompatible and/or biodegradable shaped setting material used in the above process is already known to the healthcare industry. The biocompatible and/or biodegradable shaped setting material is obtained either from tissues or proteins obtained from animal sources or natural sources or yielded by synthetic polymers.

[0035] Polymers obtained from source such as polyglucosamine, cellulose or starch can be employed as biocompatible and biodegradable membrane, preferably polyglucosamine (PGA) which is known in the art.

[0036] Polyglucosamine membrane (PGA) is of choice since it is safest on human skin and it avoids inflammatory response. The Polyglycosamine used in the above process is obtained from Vinoy Laboratories, Bangalore.

[0037] Suitable biocompatible scaffold used in the process is a biocompatible plastic and/or metal. The biocompatible plastic material is derived either by synthetic or naturally occurring polymer or copolymer.

[0038] The said synthetic polymer includes high density polyethylene, Low density polyethylene, polyurethane or polypropylene, preferably Low density polyethylene (LDPE).

[0039] The Low density polyethylene (LDPE) is preferred since the cells do not get firmly fixed to its surface thereby leaving the cells into the system easily.

[0040] The metal is biocompatible and non-toxic metal, preferably chemically inert, noncarcinogenic, and resistant to the actions of tissue fluids as well as being non-inflammatory. The plastic scaffold used in this process is flexible metal tube or sheet.

[0041] The scaffold can be of any size, shape, diameter or length complementary to the tubular structure to be repaired.

[0042] The diameter of the biocompatible scaffold tube for treating urethral stricture is in range from 22 Fr to 27 Fr.

[0043] One of the problems associated with tissue engineering for treating damages/diseases of organs involves failure to maintain their biomechanical properties like elasticity, rigidity and stability of the tissues.

[0044] The problem observed in the prior art is their failure to preserve the biomechanical properties of tissues after culturing. This leads to failures like cell occlusion, difficulties in tissue integration and shape stability.

[0045] The LDPE is biocompatible and it preserves the elasticity and stability of the tissues concerned when compared with other polymers. The above said problems have been eliminated by the methods and materials of the present invention.

**Repair of damaged tubular structure using tissue graft**

[0046] The prepared device and autograft provided with a thread is implanted at a site where urethral tissue is to be repaired or replaced. The scaffold is then removed leaving the cells inside after two to three days or after the cells begins to adhere to indigenous tissues.

[0047] Some precautions are followed to ensure that the tube is in close proximity with the raw area, to check for the presence of blood supply, and to ensure there is no contact with urine.

[0048] The surgery takes place in two steps, the first step is to get a piece of healthy urethral tissue for biopsy and the second step includes implanting the tissue graft in close proximity with the raw area and closing the urethral layers. The process consumes less

time and requires minimal hospitalization; it is economically beneficial thus overcoming the existing processes.

[0049] The cells begin to adhere within a time period of 24 hours. The biocompatible scaffold is removed back after about 3 days leaving the cells inside.

[0050] In one aspect of the invention, constructing a tissue graft for the replacing or repairing damaged or diseased urethra can be achieved by culturing cell population derived from autologus skin, but the skin cells may absorb urine during the healing period which will cause infections and will lead to longer recovering period. So the autologous urethral mucosa is found more suitable than autologus skin cells.

[0051] Present invention provides a device or auto graft which is made by culturing of autologous cell population, can be employed for replacing or repairing a damaged or diseased tubular structures and prevention of tubular stricture of organ like ureter, fallopian tube, urethra, intestine, nerves and the like.

[0052] In one embodiment, tubular structure narrow down or damage occurred by cancer or tumor can be repaired using tissue graft of the present invention.

[0053] In another embodiment, method and materials for constructing a tissue graft of the present invention can be employed to replace or repair a damaged or diseased tubular structure in animals.

[0054] In another embodiment, the tissue graft of the present invention can be employed to prevent damage or disease of a tubular structure in both the human system and the animals.

[0055] In another embodiment, the tissue graft of the present invention can be employed to replace and repair a damaged or diseased whole tubular structure using tubular graft. The tubular graft can be of any size, shape, diameter or length complementary to the vessel for repair.

[0056] In another embodiment, method and materials for constructing a tissue graft of the present invention can be used to treat and repair a specific portion of the damaged or diseased tubular structure using patch graft. The patch graft can be of any size, shape, diameter or length complementary to the vessel for repair.

[0057] The following non-limiting examples further illustrate and describe the method and materials for constructing a novel tissue graft for replacing and repairing a damaged or diseased portion of the urethra.

**Examples**

**Example 1**

**Construction of Tissue graft**

a) Isolation of autologous urethral mucosal cells

[0058] Autologous urethral mucosal cells are obtained by biopsy. The obtained cells are transported in a transport medium of Hank's phosphate buffered solution with 10x of penicillin, streptomycin and 2 to 5 mg of fungi zone and stored in thermocol box with ice packs. The transported samples are then treated with Dispase enzyme at a temperature in the range of 2° C to 4° C, to separate mucosal cells and fibrous cells.

b) Expansion of autologous urethral mucosa cells *in vitro*

[0059] The separated mucosal cells are cultured in 24 well plates for 2 to 3 days. Then, in 10 mm Petri dishes for 15-20 days. The cultured cells are expanded in 35 mm plates using keratinocyte growth medium.

c) Expansion of autologous fibroblast tissue *in vitro*

[0060] The separated fibroblasts cells are treated with collagenase enzyme at a temperature in the range of 33° C to 41° C, the connective proteins are removed and pure fibroblast cells are collected.

The obtained fibroblast cells are expanded in fibroblast growth medium and cultured in 35 mm well plate.

d) Growing autologus cells on biocompatible scaffold

[0061] The expanded autologous cells are reconstituted over a polyglucosamine membrane and it is coated on the Low density polyethylene tube (LDPE). The reconstituted cells are allowed to grow on the Low density polyethylene tube (LDPE) *in vitro* for about 1 - 2 days.

**Example 2**

**Repair of urethral stricture using Tissue graft**

[0062] The prepared tissue graft provided with thread endoscopically implanted at a site where urethral tissue is to be repaired or replaced.

[0063] The surgery takes place in two steps, the first being to take a biopsy from the healthy urethra and the second step includes implanting the tissue graft in close proximity with the raw area and closing the urethral layers. The new cells grown attach themselves snugly to the natural cells.

[0064] The cells then begin to adhere with indigenous urethral tissues within a time period of 24 hours. The Low density polyethylene tube (LDPE) is removed back after about 3 days leaving the cells inside.

**Results/Discussions**

The obtained clinical trial output is discussed below.

**a. Bladder Ultrasound Study**

[0065] To measure the post voidal residual urine volume (PVR) Bladder Ultrasound or bladder scan is used and the results are shown in the Fig.1. After surgical repair and reconstruction of the urethra the post voidal residual urine volume (PVR) returns to the normal level of< 100mLs. Preoperative results show a high volume of post voidal residual urine volume (PVR) of about 200 – 600 mLs due to the strictures in the urethra which hinders the passage of the urine and retains urine in the bladder. Post voidal residual urine volume (PVR) of greater than 100 mLs indicates incomplete bladder emptying.

**b. Uroflowmetric assessments**

[0066] Uroflowmetry measures urine voided per unit time, which is usually expressed as milliliters per second. During the Uroflow measurement voided volume, rate of flow and the voiding time are calculated and regarded to be the most clinically useful tool for both screening and following patients.

[0067] To measure the voiding volume Uroflowmetry is performed and the results are displayed as shown in the Fig.2 and Table 1. Table.1 depicts the picture of Uroflowmetry which shows the change of percentage in the voiding volume of the patient during the successive visits. The volume of the urine expelled before the treatment of the urethral stricture is in the range of 150 – 250 mLs which gradually increased after the treatment and reached a steady volume of 300 mLs during the successive visits of the patient as shown in the Fig.2.

[0068] The rate of flow of urine and the voiding time is assessed by the Uroflowmetry and the results are shown in the Table.2 and Table 3.

[0069] Table.2 shows the result of Uroflowmetry which illustrates the percentage of the change in the rate of flow of the urine with respective to the number of visits of the patient.

Table.3 shows the result of Uroflowmetry depicting the pattern of fluctuations in the voiding time of the patients with respect to the number of visits of the patient.

[0070] These tabular representations illustrate the increase of rate of flow and the voiding time of the urine before and after the treatment and showed positive results which is indicated by the increase in the rate of flow and voiding time values.

**Table 1:** Voiding Volume

| Visit | Avg | % change |
|---|---|---|
| 2 | 153.8 | |
| 4 | 244.1 | 159.4 |
| 5 | 301.2 | 196.7 |
| 6 | 300.05 | 196.1 |

**Table 2:** Rate of Flow

| Visit | Avg | % change |
|---|---|---|
| 2 | 4.6 | |
| 4 | 93.4 | 339.8 |
| 5 | 24.9 | 199.4 |
| 6 | 11.7 | 144.3 |

**Table 3:** Voiding Time

| Visit | Avg | % change |
|---|---|---|
| 2 | 70 | |
| 4 | 21 | -75 |
| 5 | 17 | -13.7 |
| 6 | 69 | 152.2 |

**C. Immunohistochemical analysis**

[0071] Immunohistochemical analysis is widely used in the diagnosis of abnormal cells such as cancer cells. Reverse transcription polymerase chain reaction (rt-PCR), a sensitive method used for the detection of any abnormal cells and hence used as a measure of gene expression. Keratin expression of the normal skin keratinocytes and newly cultured cells is examined using K10 and K17 as markers. Results of these studies revealed that there are no abnormal changes in the newly cultured cells and the post operative analysis is successful.

[0072] It is apparent that there have been provided in accordance with the present invention a device and autologous urethral mucosal tissue graft composition, a method of engineering the latter for use in treating the urethral stricture, which fully satisfies the objectives and the advantages set forth above.

[0073] The IPSS (International Prostate Symptom Score) of the patients also reflects the same positive improvements which have been an evidence for utilizing autologous urethral mucosa cells for tissue engineering by culturing them on LDP tubes.

**What we claimed is:**

1. A novel apparatus for use in repairing or replacing damaged or diseased tubular structure and repair and prevention of tubular stricture in mammals, said apparatus comprising a biocompatible scaffold encircled by a biocompatible and/or biodegradable shaped setting material with grown cells.

2. A novel tissue graft in tubular form or in patch graft form comprising autologous epithelial cells for use in repairing or replacing damaged or diseased tubular structure and repair and prevention of tubular stricture in mammals; the said graft being set in a naturally derived or synthetic biocompatible or biodegradeable scaffold.

3. An apparatus as claimed in claim 1, wherein the said biocompatible scaffold comprises biocompatible polymer or biocompatible, non toxic metal.

4. An apparatus as claimed in any of the preceding claims, wherein the said biocompatible polymer comprises synthetic or naturally derived polymer or copolymer.

5. An apparatus as claimed in any of the preceding claims, wherein the said biocompatible and/or biodegradable shaped setting material is either obtained from natural sources or synthetic route.

6. An apparatus as claimed in any of the preceding claims, wherein the said biocompatible scaffold is provided with a synthetic or natural thread, rope, belt and the like.

7. An apparatus as claimed in any of the preceding claims, wherein the said grown cells are autologous cells.

8. An apparatus as claimed in any of the preceding claims, wherein the said autologous cells comprise urothelial cells or skin cells.

9. An apparatus as claimed in any of the preceding claims, wherein the said urothelial cell is autologous urethral mucosa.

10. An apparatus as claimed in any of the preceding claims, wherein the said urothelial cells comprise substantially autologous fibroblast cells.

11. An apparatus as claimed in any of the preceding claims, wherein the said tubular structure is selected from the group consisting of ureter, fallopian tube, urethra, intestine and nerves.

12. An apparatus as claimed in any of the preceding claims, wherein the said tubular structure is urethra.

13. An apparatus with tissue graft as claimed in any of the preceding claims, wherein the biomechanical properties of the tissue concerned are preserved.

14. A novel tissue graft in tubular form or as patch for use in repairing or replacing damaged or diseased tubular structure and prevention of tubular stricture, said tissue graft comprising a biocompatible scaffold encircled by a biocompatible and/or biodegradable shaped setting material with grown cells.

15. A tissue graft as claimed in claims 13, wherein the said biocompatible polymer comprises synthetic or naturally derived polymer or copolymer.

16. A tissue graft construct as claimed in claims 13 or 14, wherein the said biocompatible and/or biodegradable shaped setting material is either obtained from natural sources or synthetic route.

17. A tissue graft as claimed in claims 13, 14 or 15, where in the said biocompatible scaffold is provided with a synthetic or natural thread, rope, belt and the like.

9. An apparatus as claimed in any of the preceding claims, wherein the said urothelial cell is autologous urethral mucosa.

10. An apparatus as claimed in any of the preceding claims, wherein the said urothelial cells comprise substantially autologous fibroblast cells.

11. An apparatus as claimed in any of the preceding claims, wherein the said tubular structure is selected from the group consisting of ureter, fallopian tube, urethra, intestine and nerves.

12. An apparatus as claimed in any of the preceding claims, wherein the said tubular structure is urethra.

13. An apparatus with tissue graft as claimed in any of the preceding claims, wherein the biomechanical properties of the tissue concerned are preserved.

14. A novel tissue graft in tubular form or as patch for use in repairing or replacing damaged or diseased tubular structure and prevention of tubular stricture, said tissue graft comprising a biocompatible scaffold encircled by a biocompatible and/or biodegradable shaped setting material with grown cells.

15. A tissue graft as claimed in claims 13, wherein the said biocompatible polymer comprises synthetic or naturally derived polymer or copolymer.

16. A tissue graft construct as claimed in claims 13 or 14, wherein the said biocompatible and/or biodegradable shaped setting material is either obtained from natural sources or synthetic route.

17. A tissue graft as claimed in claims 13, 14 or 15, where in the said biocompatible scaffold is provided with a synthetic or natural thread, rope, belt and the like.

18. A tissue graft as claimed in claims 13, 14, 15 or 16, wherein the said grown cells are autologous cells.

19. A tissue graft as claimed in claims 13, 14, 15, 16 or 17, wherein the said autologous cells comprise urothelial cells or skin cells.

20. A tissue graft as claimed in claims 13, 14, 15, 16, 17 or 18, wherein the said urothelial cell is autologous urethral mucosa.

21. A tissue graft construct as claimed in claims 13, 14, 15, 16, 17, 18 or 19, wherein the said urothelial cells comprise substantially autologous fibroblast cells.

22. A tissue graft construct as claimed in claims 13, 14, 15, 16, 17, 18, 19, or 20, where in the said tubular structure is selected from the group consisting of ureter, fallopian tube, urethra, intestine and nerves.

23. A tissue graft construct as claimed in any of the preceding claims, wherein the said tubular structure is urethra.

24. A method of tissue engineering autologous cells in tubular form or as patch for use in repairing or replacing damaged or diseased tubular structure and prevention of tubular stricture, comprising:
   a) providing a biocompatible scaffold;
   b) growing autologous cells on the biocompatible scaffold, to thereby create a tissue structure, or segment thereof, suitable for implantation at a site where tubular structure is to be replaced or repaired.

25. A method of tissue engineering as claimed in claim 23, wherein the said biocompatible scaffold comprises a biocompatible polymer or biocompatible, non toxic metal.

26. A method of tissue engineering as claimed in claims 23 or 24, wherein the said biocompatible polymer comprises synthetic or naturally derived polymer or copolymer.

27. A method of tissue engineering as claimed in claims 23, 24 or 25, wherein the said biocompatible scaffold is coated with a biocompatible and/or biodegradable shaped setting material.

28. A method of tissue engineering as claimed in claims 23, 24, 25 or 26 wherein the said biocompatible scaffold is provided with a synthetic or natural thread, rope, belt and the like.

29. A method of tissue engineering as claimed in claims 23, 24, 25, 26 or 27, wherein the said autologous cells comprise urothelial cells or skin cells.

30. A method of tissue engineering as claimed in claims 23, 24, 25, 26, 27 or 28, wherein the said urothelial cell is autologous urethral mucosa.

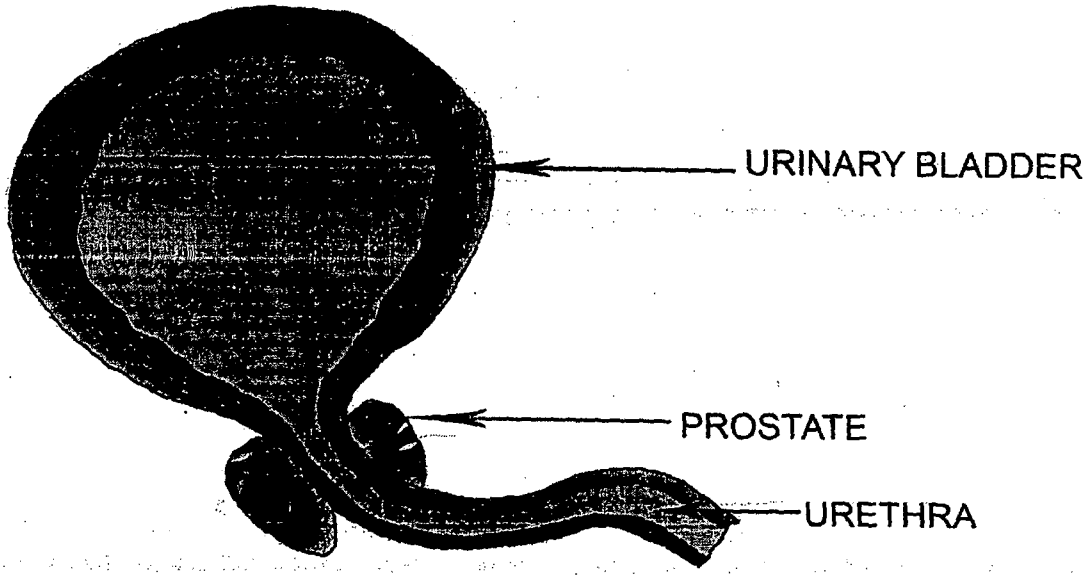
31. A method of tissue engineering as claimed in claims 23, 24, 25, 26, 27, 28 or 29, wherein the said urothelial cells comprises substantially autologous fibroblast cells.

32. A method of tissue engineering as claimed in claims 23, 24, 25, 26, 27, 28, 29 or 30, wherein the said organ or tissue to be regenerated is selected from the group consisting of ureter, fallopian tube, urethra, intestine and nerves.

33. A method of tissue engineering as claimed in claims 23, 24, 25, 26, 27, 28, 29, 30 or 31, wherein the said tubular structure is urethra.

34. A tissue graft for use in replacing or repairing a damaged or diseased tubular structure and prevention of tubular stricture in animals, particularly mammals and/or humans as substantially described herein in the accompanying description.

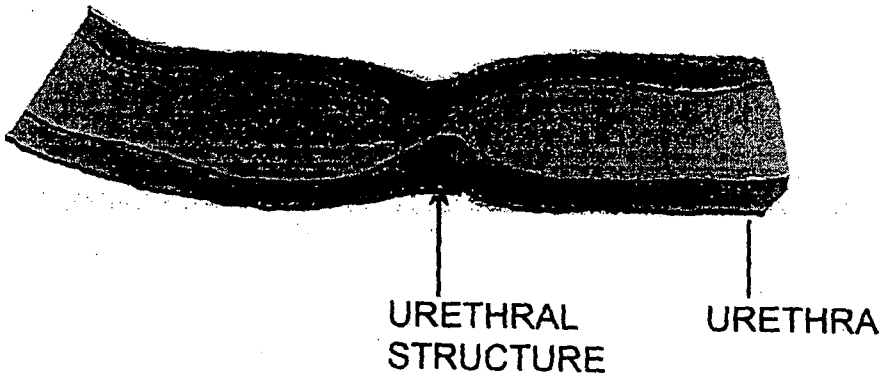
FIGURE 1.1




STRUCTURE OF THE MALE URETHRA

FIGURE 1.2

STRICTURE IN THE MALE URETHRA



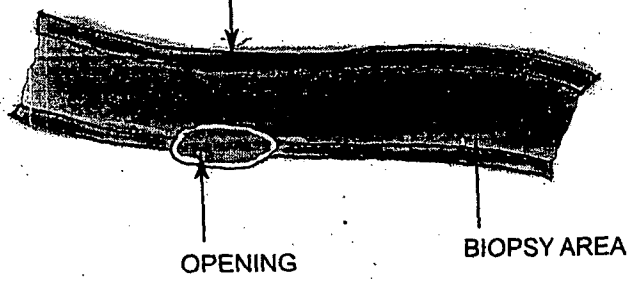



FIGURE 2.1

EXCISION OF THE MUCOSA

OPENING OF THE URETHRA SO THAT URINE PSSES THROUGH THIS OPENING

FIGURE 2.2

CULTURE OF AUTOLOGOUS URETHRAL MUCOSAL TISSUE.

TISSUE EXPANDED

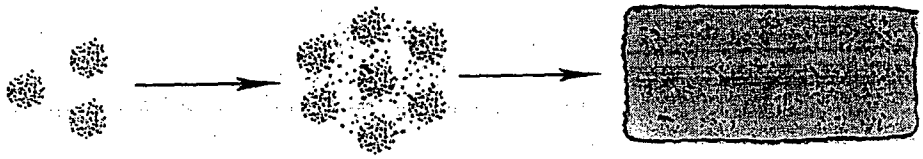
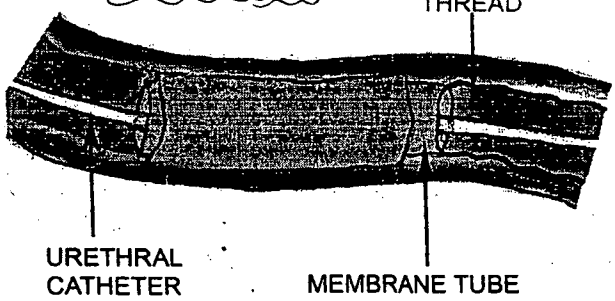



FIGURE 2.3

NOVEL METHOD OF TUBULAR CULTURE OF THE AUTOLOGOUS URETHRAL MUCOSA

LOW DENSITY POLYTHENE TUBE LDP

FIGURE 3.1

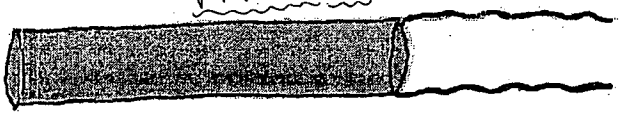



WITH CELLS WRAPPED ON THE LDP POSITINED WITH BLACK SILK.

AFTER 2 DAYS- THE LDP IS REMOVED WITH THE HELP OF BLACK SILK THREAD

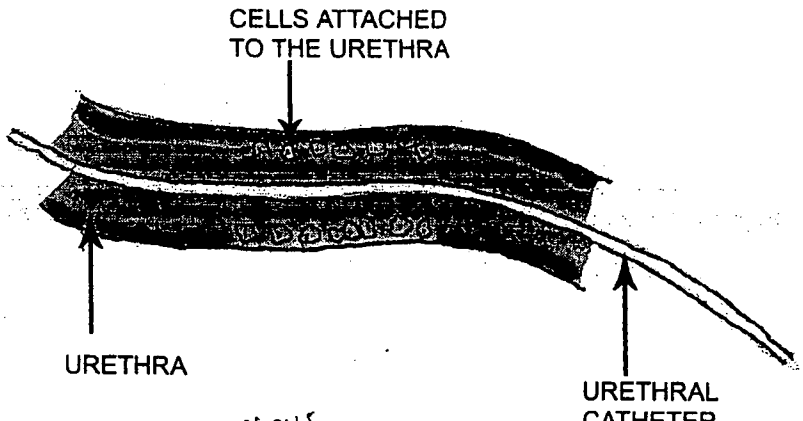
PLACEMENT OF JUBULAR GRAFT IN THE AFFECTED REGION OF THE URETHRA

FIGURE 3.2



LDP TUBE WITH LACK SILK THREAD

FIGURE 3.3




ATTACHMENT OF AUTOLOGOUS URETHRAL MUCOSAL TISSUE IN A TUBULAR FORM IN THE AFFECTED REGION