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STUDY PROTOCOL

SAFETY, TOLERABILITY AND EFFICACY EVALUATION OF IMMEDIATE TOTAL WRAPPING WITH BIOLOGICAL MESH IMPLANT-BASED BREAST RECONSTRUCTION: AN UNDER-ESTIMATED SUBCUTANEOUS APPROACH WITH "BIOLOGICAL TEXTURIZATION" PROSTHESES PRECLINICAL ANIMAL STUDY

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ABSTRACT

PURPOSE: The aim of this study is to analyze tissue reaction after implantation of three different types of mini-silicone sub-cutaneous round prosthesis (smooth, texturized and polyurethane) with and without an envelope of APMs, using a rat experimental model.

METHODS: One hundred eighty six female Wistar rats divided into 13 groups went through an experimental

procedure split into three phases: the 1st phase consisted of implanting and controlling of APMs; the 2nd one involved implanting smooth, texturized, polyurethane silicone prostheses; the 3rd and last phase included the use of APMs completely enveloped with silicone prostheses.

RESULTS: At 3, 6 and 24 weeks postoperatively, the samples were explanted and subjected to clinical, histological and immunohistochemical evaluation with excellent outcome.

CONCLUSION: Our subcutaneous biological complete wrapping approach perfectly met the criteria of efficacy and safety in our animal model.

KEY WORDS: Breast Reconstruction, Implants, Total wrapping of implants, Acellular Pericardium Matrix, Subcutaneous approach, Capsular contracture

Introduction

Breast cancer (BC) remains the most frequent diagnosed malignancy in Western and developed countries and is a major cause of illness in women, with an estimated incidence of 300.000 new cases diagnosed in 2013^(1,2). In recent years BC was the most common cancer amongst Italian women, with 48.000 new diagnosed cases⁽³⁾. Approximately 35-40% of women diagnosed with BC undergo a total mastectomy, a trend that is in constant increase. Less than 33% of the patients who meet the criteria undergo breast reconstruction (BR) after mastectomy⁽⁴⁾.

Early detection and identification of subjects at high risk for developing cancer in familial-hereditary status have largely contributed to increase life expectancy (overall survival – OS) and the disease-free survival (DFS). Oncological and breast studies are now moving their efforts on how to ameliorate the patients' quality of life (QoL). This is quite a delicate process is closely connected to the patient's individual body image and integrity; not to mention that, BC treatment often leads to mutilation and destruction of breast shape, with additional consequences on self-esteem. For this reason, in women treated with mastectomy, BR has become an essential element of the entire therapeutic procedure, being requested by an increasingly large number of patients, as the goal of BR is to restore the patient's body image, to improve femininity and maintain the QoL without affecting the prognosis or the detection of recurrences.

BR can be performed with the use of either implants or autologous tissue. The choice of either technique is dictated by a variety of factors but the final decision is often made in accordance with the patient's preference. The first option for an implant-based BR (IB-BR) includes a two-



stage reconstruction with a tissue expander, followed by a permanent implant and most of the time with intervening adjuvant therapy - a process referred as delayed breast reconstruction (D-BR). Another alternative is an immediate-at the time of the mastectomy- single stage reconstruction with an implant (IIB-BR) and the last one is a reconstruction with the combination of implant and autologous tissue. Immediate breast reconstruction has been in constant increase since 2005 with an associated rise in implant-based reconstruction⁽⁵⁾. Prosthetic BR has the advantages of shorter procedure time, hospital stay and recovery as well as being less expensive⁽⁶⁾ and more importantly it does not set the requirement to get an additional donor site associated with an autologous reconstruction⁽⁷⁾. The IIB-BR, compared to the reconstruction in two stages, allows an immediate aesthetic result, improving the patient's compliance, and attains total restoration of mammary volume and shape⁽⁸⁾; nevertheless, it is appropriate for a small number of patients with a small breast and absence of ptosis, good quality of skin and muscle tissue, that will definitely allow an immediate placement of the implant. The disadvantage of the IIB-BR approach is that the aesthetic outcome does not tend to be as good as after a two-stage reconstruction and, in many cases, a second procedure may be necessary.

Biological meshes are medical devices designed for local implantation, which provide soft tissue reinforcement. Surgical breast reconstruction (BR) is a standard approach typically performed in women after mastectomy treatment for breast cancer (BC). While autologous BR is still considered the procedure of choice for immediate BR (I-BR), there has been a shift towards implant-based BR (IB-BR) during the past few years. The proven safety of silicone breast implants and the development of biological matrices have contributed to the growing popularity of the immediate IB-BR (IIB-BR) approach. In IB-BR, the implant is typically sited in a sub-pectoral pocket; no tissue from the pectoral muscle is usually available in the lower lateral part to provide additional cover or to support the implant itself. This can lead to increased implant palpability with a lack of support and subsequent skin erosion and capsular contracture (CC). CC is described as the formation of a fibrous capsule around the implant, which may contract and compress the implant as it progressively thickens, resulting in a hard breast with deformed contouring of the surrounding skin. The introduction of a biological mesh into breast surgery has contributed to resolve surgical restrictions in IB-BR, as it allows the surgeon to cover the

implant even when native skin cover is insufficient. Based on clinical and experimental data, the use of a biological mesh as a sheath around implants may lead to lesser capsular contracture acting.

Breast implants are two types, saline and silicone gel; they have an outside shell made from solid silicone and can be either smooth or textured. Each type of implant can be round shape or anatomically adaptive. The human body's immune response to a surgically installed foreign object-breast implant is to contain it with scar tissue capsules of tightly bonded collagen fibers, in order to maintain the integrity of the human body by isolating the foreign object, and so to tolerate its presence. This normal capsular tissue cover is the key point of the treatment in terms of actual treatment. It closes off and grants the right position onto the body. Unfortunately, the body reaction can be aggressive and form stiffer and thicker capsules. In some cases, this may be associated with pain, soft tissue irritation via capsular contracture (CC) and lead, from a cosmetic point of view, to an undesirable appearance to the breast⁽⁹⁾. CC is the most common complication associated with IB-BR after mastectomy for cancer (21.8% at 1 year and 34% at 5 years)⁽¹⁰⁾ and its occurrence is multifactorial. CC rates in IIB-BR have been reported to oscillate between 20%⁽¹¹⁾ to 40.4%⁽¹²⁾. Additional corrective surgeries may be required to remove the capsule, resulting in amplified costs for the patient and the healthcare system. Based on clinical and experimental data, the use of acellular pericardial matrices (APMs) for IB-BR appears to be associated with a lower incidence of CC compared to standard reconstruction. APMs are made of extracellular matrix (ECM) proteins and they work as a useful structure for migration, adhesion and cellular proliferation. It follows the developing of connective tissue^(13,14).

Methods

Animals

One hundred and eighty-six adult female Wistar rats, each of them weighing 180-220 g, were purchased from the "Harlan Laboratories, Italy". The rats were randomly numbered and divided into thirteen groups, twelve study groups of 15 rats each and one control group of 6 rats.

Implantation materials

For this study, three different types of biological meshes were used. Bioripar "Dry" (BRD) and Bioripar "Wet" (BRW) - was fabricated by ASSUT-EUROPE (Rome, Italy). Tutomesh (TM) was fabricated by Tutogen Medical GmbH (Neunkirchen am Brand, Germany) and were purchased

from ASSUT-EROPE (Rome, Italy). In total, we used five TM, five BRW and two BRD meshes. The BRD mesh has been abandoned and not been utilized during phase 3 because of its reduced practicality (increased rigidity-low flexibility make hard surgical maneuvers) (Table 1 and Figure 1). For this study, one hundred thirty five silicone gel-filled mini-prosthetic devices, fabricated by SILIMED-TM (Company, São Paulo, Brazil) and purchased from VEDISE-HOSPITAL (Rome, Italy), we used. The prostheses were of three different types: smooth (S), textured (T) and polyurethane (P) (Table 2 and Figure 2).

Brand Name/ Manufacturer	Bioripar “Dry” (ASSUT Europe, Rome, Italy)	Bioripar “Wet” (ASSUT Europe, Rome, Italy)	Tutomes® (Tutogen Medical GmbH, Nürnberg, Germany)
Species of Origin	Xenogenic	Xenogenic	Xenogenic
Animal and Tissue Source	Adult Bovine Pericardi- um	Adult Bovine Peri- cardium	Adult Bovine Pericardium
Collagen			I
Decellulariza- tion process- ing	a.		
	a.		
	n.a.		
Cross-linker	None	None	None
Need for refrigeration	NO	NO	NO
Rehydrata- tion require- ment	Yes	NO	Yes
Sterilization method	Salt solution	g-irradiation	g-irradiation
Thickness	0.2-0.6mm	0.4-0.7mm	0.5 mm

a.: available, n.a.: not available, mm: millimeter

Table 1: Overview of biological meshes used.



Figure 1: Biological APMs used. (A) Tutomes®; (B) Bioripar® “Dry”; (C) Bioripar® “Wet”.

Brand Name/ Manufacturer	SILIMED-TM (Company, São Paulo, Brazil)		
Type	Smooth	Textured	Polyurethane
Round-based <_>	2.0 x 0.85 cm	2.2 x 0.85 cm	2.3 x 0.85 cm
Volume (VOL)	2 cm ³		

Table 2: Details of Gel Filled mini prostheses used.



Figure 2: SILIMED Gel Filled mini-prosthesis used. (A) Smooth; (B) Textured; (C) Polyurethane.

Experimental groups

As previously described, thirteen groups of rats were evaluated in this study:

1. One Control Group: 6 animals were randomized to receive one surgical procedure only;
2. Three Mesh Groups: 45 animals were randomized to receive only a biological mesh - divided in three groups each of which consisted of 15 rats (Mesh Group BRD, Mesh Group BRW and Mesh Group TM);
3. Three Prosthesis Groups: 45 animals were randomized to receive only a silicone gel-filled mini-prosthesis – divided into three groups each which consisted of 15 rats (Smooth Prosthesis Group (SPG), Texture Prosthesis Group (TPG) and Polyurethane Prosthesis Group);
4. Three Prosthesis-Bio-repair “Wet” Groups: 45 animals were randomized to receive silicone gel filled mini-prosthesis with an overlay of a Bio-repair “Wet” biological mesh, divided into three groups each of which consisted of 15 rats (Smooth Prosthesis-Bio-repair “Wet”

Group, Texture Prosthesis-Bio-repair “Wet” Group and Polyurethane-Bio-repair “Wet” Group);

5. Three Prosthesis-Tutomesh Groups: 45 rats were randomized to receive silicone gel filled mini-prosthesis with an overlay of a Tutomesh biological mesh-divided into three groups each of which consisted of 15 rats (Smooth Prosthesis-Tutomesh Group, Texture Prosthesis-Tutomesh Group and Polyurethane-Tutomesh Group).

Each group of animals was randomly split into three subgroups of 2 rats for the Control Group and 5 animals for the experimental groups. The animals were sacrificed at the following time: 3, 6 and 24 weeks.

Surgical Procedures

All surgical procedures were performed under general anesthesia. Each animal was anaesthetized by intra-abdominal injection of tiletamine/zolazepam (50 mg/Kg) (Zoletil 100, Virbac, Italy) associated to xylazine (15mg/Kg) (Rompun, Bayer, Italy). Under anesthesia, each animal was placed in the prone position. After epilation of the dorsum, the skin was disinfected with a povidone-iodine solution. The surgical sites were located 3 cm above the hind limbs insertion in the lumbar region. After making a 4 cm transverse skin incision, a tunnel was created above the panniculus carnosus, up to the shoulder blades. The fascia was kept intact. The defects were randomly assigned to receive mesh or mini-prosthesis or mini-prosthesis and mesh. A 7x3 cm mesh was placed into the tunnel, in the rats of mesh groups. In the rats belonging to the groups of mini-prosthesis, with or without mesh, the device was inserted through the incision and placed into a pocket between scapulae, approximately 3 cm apart from the incision. The overlying subcutaneous tissue and skin were closed using skin staplers (Appose™ -Single Use Skin Stapler- COVIDIEN PRODUCTS by Medtronic, Regular Staple -width/crow 4.8 mm and leg length 3.4 mm) (Figure 3).



Figure 3: Surgical procedures. (A) epilation on the dorsum; (B) surgical site located 3 cm above the lumbar region; (C) placement the mesh in the panniculus carnosus of the rats of mesh groups; (D) the overlying subcutaneous tissue and skin were closed using skin staplers.

Ethics Statements

This study was performed at the Station for Animal Technology (SAT) of the Tor Vergata University of Rome, Italy. The experimental protocol was approved by the Institutional Animal Care and Use Committee in according to the Legislative Decree (L.D.) 116/92. All surgical procedures and animal handling were conducted in accordance with the guidelines of the Guide for Care and Use of Laboratory Animals – 8th Edition, released by the Institute for Laboratory Animal Research (National Academies Press, 2011) and according to the ethical principles of the National Committee of Bioethics (Comitato Nazionale per la Bioetica - CNB) Veterinarian security & safety management, according and due to art. 6 of the L.D. 116/92.

Postoperative Care

Animals were housed under standard cage condition of light-dark cycles (12/12h) and food-water access *ad libitum*. Ambient temperature was of 20-/+2°C, relative air humidity was approx. 5.5%. Gross visual observations were made daily recording general condition parameters (appearance, attitude, appetite, and hydration), body weight and food consumption, as well as the surgical incision site for clinical signs of infection, seroma, hematoma, staples breakdown, wound dehiscence, position of the implants, mesh rejection, erosion and exposure of the mini-prostheses. After 14 days we evaluated the incision healing process and we removed the staple using an appropriate skin staple remover (removal staple time: 14 days).

Experimental Time Table

The interventions have been chronologically set up into three phases.

Phase 1 - Control Group and Implanting of APMs alone (Figure-4):

- Group 1: Control Group - 6 rats;
- Group 2: Mesh A Group (BRD) - 15 rats;
- Group 3: Mesh B Group (TM) - 15 rats;
- Group 4: Mesh Group C (BRW) - 15 rats.



Figure 4: Control Group and Implanting of APMs alone. (A-C) Implant sequence of the single biological mesh.

Phase 2 - Implanting of Silicone Prostheses only (smooth, texturized and polyurethane silicone prostheses) (Figure-5):

- Group 5: Smooth silicone prostheses Group - 15rats;
- Group 6: Texturized silicone prostheses Group - 15 rats;
- Group 7: Polyurethane silicone prostheses Group - 15 rats.

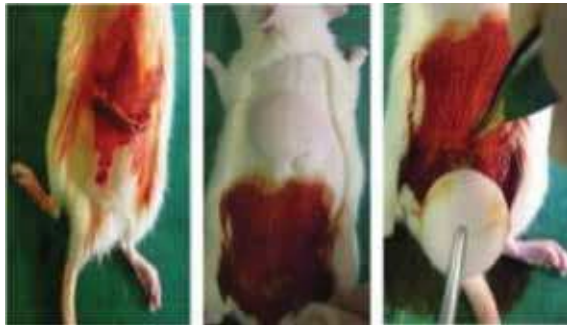


Figure 5: Implanting of polyurethane silicon prostheses alone. (A-C) Implant sequence of the single prostheses.

Phase 3 - Implanting of Silicone Prostheses totally covered with Type B & C Biological Mesh: Groups 8, 9, 10, 11, 12, 13 (Figures 6 and 7).

- Group 8: Smooth silicone prostheses Group totally covered by mesh B - 15 rats;
- Group 9: Texturized silicone prostheses Group totally covered by mesh B -15 rats;
- Group 10: Polyurethane silicone prostheses Group totally covered by mesh B - 15 rats.
- Group 11: Smooth silicone prostheses Group totally covered by mesh C - 15rats;
- Group 12: Texturized silicone prostheses Group totally covered by mesh C - 15 rats;
- Group 13: Polyurethane silicone prostheses Group totally covered by mesh C - 15 rats.

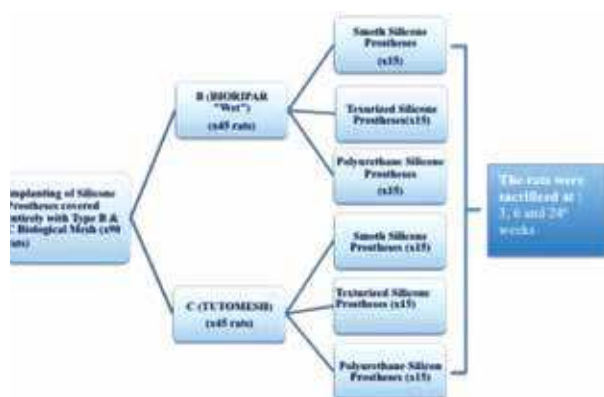


Figure 6: Representative diagram of groups with silicone prostheses covered entirely with APMs.



Figure 7: Implanting of silicone prostheses covered entirely with APMs. (A) Silicone prostheses totally covered with APMs before the implantation; (B) placement the prostheses in the dorsal region of the rats.

All animals/group were sacrificed at 3, 6 and 24 weeks after surgical procedure.

Macroscopic Examination

Before sacrifice, each animal was examined by inspection for evidence of epidermal ulceration and dermal inflammation. At the time of sacrifice, rats were examined for evidence of seroma and eventual implant infection before sampling.

Histopathologic Examination

For tissue evaluation, biological mesh or silicone prostheses alone or covered by mesh were removed “en-bloc” including overhead skin. For sampling, the mesh, together with at least 3 cm of surrounding tissue, the fascia-capsule and the skin-capsule, were harvested and stored for histopathological processing. Retrieved samples with a 2-cm piece of surrounding tissue were trimmed and fixed in neutral buffered formaldehyde for 48 hours and then embedded in paraffin for histological evaluation (Figure 8).

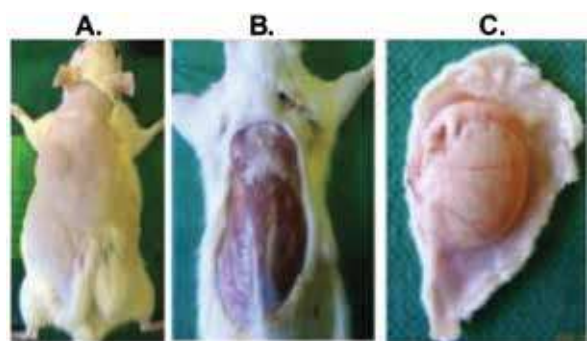


Figure 8: Silicone prostheses covered by mesh removed “en-bloc” including overhead skin at 24 weeks of explanation. (A) dorsal region before the removal; (B) dorsal region after the removal; (C) the samples including the mesh, graft host tissue interface and adjacent host tissue with panniculus carnosus, subcutaneous tissue, skin and silicon prostheses.

Rats were dissected, skin and fascia were removed, and the axillary lymph nodes (AxLNs) were obtained for



histopathological processing.

The purpose of this trial was to evaluate the development of phenomena, such as:

- Epidermal and dermal changes (epidermal ulceration, epidermal thickness, dermal inflammation, dermal collagen changes);
- Nature of host response (incorporation, encapsulation, resorption, rejection and mixed response);
- Inflammatory reactions, both acute and chronic, of a foreign body;
- Peri-prostheses capsule changes (composition, capsule thickness);
- Vascular changes and Neovascularization.

Blood Collection from the Orbital Sinus

In this study we also evaluated the blood reaction referred to the different types of mesh and implants (single biological mesh, single silicone prostheses, silicone prostheses totally covered by biological mesh). For this reason, blood collection was obtained after retro-orbital sinus puncture under general anesthesia.

For determination of haematological parameters, 20 µl of whole blood was collected in K2EDTA micro-vacutainers (Boston, Dickinson and Company, USA) and the samples were analysed using the commercially available automated cell counter "Drew3" (BPC BioSed, Italy). For cytomorphological examination, each sample from peripheral blood smears was prepared using the differential staining Diff-Quick (Dade SpA, Italy) and analysed under optical microscopy. For serum protein electrophoresis blood samples were collected in SST micro-vacutainers (Serum Separator Tube; Boston, Dickinson and Company) and centrifuged in a micro-centrifuge (5415R model; Eppendorf, Italy) at 13.000 rpm for 7 mins so as to isolate the serum and applied to cellulose polvacetate electrophoresis strips, for the automatic analyser Simply Phor (BPC BioSed, Italy).

Discussion

The aim of this in vivo protocol was to study, in a prospective way, the feasibility, tolerability and safety of a single BR treatment, using a biological mesh totally covering a silicone prostheses positioned under skin and over muscle. To the best of our knowledge, this is the first report on an experimental complete wrapping of three different types of silicone gel-filled implants with a two different types of APMs. The acellular matrices are opening the way for a new approach to HBBR and the customization of treatment.

Trial Status: This study has been completed.

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Enrolment: 186 rats

Study Start Date: April 2014

Study Completion Date: May 2015

Primary Completion Date: November 2015 (Final data collection date for primary outcome measure)

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Conflict of interest: The authors declare no conflict of interest.

Author Contributions: Prof Piero Rossi, Dr Dimitrios Varvaras and Prof. Oreste Claudio Buonomo designed the study and performed all animal surgeries. Prof Augusto Orlandi and Dr Alessandra Bielli performed histological analysis. Prof Giuseppe Petrella directed the study. Prof. Maurizio Mattei and Dr Roberta Bernardini analysed all data. Dr Dimitrios Varvaras composed the manuscript together with all co-authors.

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