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ORIGINAL ARTICLE

Cell-free biomimetic polyurethane-based scaffold for breast reconstruction following non-malignant lesion resection. A first-in-human study

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Abstract

Background Based on the volume of tissue removed, conservative surgery (BCS) cannot always guarantee satisfactory cosmetic results, unless resorting to more complex oncoplastic approaches. Investigating an alternative to optimize aesthetic outcomes minimizing surgical complexity, was the purpose of this study. We assessed an innovative surgical procedure based on the use of a biomimetic polyurethane-based scaffold intended for regenerating soft-tissue resembling fat, in patients undergoing BCS for non-malignant breast lesions. Safety and performance of the scaffold, and safety and feasibility of the entire implant procedure were evaluated.

Methods A volunteer sample of 15 female patients underwent lumpectomy with immediate device positioning, performing seven study visits with six-month follow-up. We evaluated incidence of adverse events (AEs), changes in breast appearance (using photographs and anthropomorphic measurements), interference with ultrasound and MRI (assessed by two independent investigators), investigator's satisfaction (through a VAS scale), patient's pain (through a VAS scale) and quality of life (QoL) (using the BREAST-Q© questionnaire). Data reported are the results of the interim analysis on the first 5 patients.

Results No AEs were device related nor serious. Breast appearance was unaltered and the device did not interference with imaging. High investigator's satisfaction, minimal post-operative pain and positive impact on QoL were also detected.

Conclusions Albeit on a limited number of patients, data showed positive outcomes both in terms of safety and performance, paving the way to an innovative breast reconstructive approach with a potential remarkable impact on clinical application of tissue engineering.

Trial registration ClinicalTrials.gov (NCT04131972, October 18, 2019).

Keywords Breast reconstruction · Lumpectomy · Aesthetic outcome · Tissue engineering · Scaffold

Introduction

Nowadays, satisfactory aesthetic results together with oncological radicality are the main goals of breast conserving surgery (BCS) [1, 2]. However, traditional BCS may not always be able to achieve both of them in an equally satisfactory way [3–5], since the aesthetic result is correlated to the lesion/breast ratio and is influenced by the location of the lesion. A traditional BCS in case of large lesions in small breasts and/or in unfavorable locations can result in predictable breast deformities. Accordingly, oncoplastic breast surgery (OBS) has been developed [6], but compared to traditional BCS, OBS is technically more demanding, time consuming, and frequently bilateral [7]. Therefore, even if the currently available surgical options are valid, having an alternative technique that optimizes aesthetic results minimizing surgical invasiveness and complexity, is highly desirable. Investigating such an alternative was the purpose of this first-in-human (FIH) pilot study, in which we assessed feasibility and safety of an innovative surgical procedure based on the immediate implantation of a bioactive scaffold in patients undergoing BCS for non-malignant lesions. Even if this device is underway for future use in breast cancer

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(BC) patients eligible for OBS, in this pilot study we tested it in this specific patient population, which did not require adjuvant therapies, to have data more clearly device related. We believe that combining BCS with the unique characteristics of this scaffold, could significantly improve patient's quality of life (QoL) by means of an easy-to-adopt and potentially cost-saving procedure. The data reported herein are the results of the interim analysis on the first 5 patients enrolled, followed up for 6 months.

Patients and methods

Study design and device description

This is a pre-market, single-center, interventional, open-label, non-comparative, feasibility/pilot FIH study. The purpose is to acquire preliminary information on an innovative device and surgical technique to design an adequate development plan. It was conducted in conformity with the ethical principles of the Declaration of Helsinki and the Good Clinical Practice principles, and was approved by the local hospital Ethical Committee (Protocol # 51,965, 11 October 2018), and the Italian Ministry of Health (0,066,926–05/12/2018-DGDMF-MDS-P). A volunteer sample of fifteen female patients, aged 20–85 years, with a proven diagnosis of non-malignant lesion up to 200 cubic centimeters in volume, eligible for lumpectomy, was selected according to criteria listed in Fig. 1. The device (i.e. REGENERA™,Tensive Srl, Milan, Italy), which is supplied sterile for single use only in a double primary package, is a 70 cubic centimeters oval-shaped cross-linked poly(urethane-ester-ether)-based matrix, characterized by an interconnected open-pore structure (pore diameter from 0.3 to 6.5 mm), where a 3D network of channels propagates along X, Y and Z axes. Its shape and structure (Fig. 2), are designed to minimize friction against the surrounding tissue and to allow cell infiltration [8, 9]. Notably, the polymeric component occupies less than 3% of its volume. The remaining space, created by pores and channels, provides a suitable architecture for tissue ingrowth, supporting vascularized soft-tissue self-organization. Its mechanical properties closely match those of human adipose tissue [10], mimic the native micro-environment, and enable mechanically induced cell signaling for tissue regeneration [11, 12]. In consideration of the completely innovative nature of the device, a literature review for each constituent component and different in vitro and in vivo animal tests on the device in its final form [13] were performed before undertaking the study in humans, to assess its safety profile and biocompatibility in line with the endpoints identified according to its categorization by ISO 10993-1 [Category: Implant medical device;

Inclusion criteria

- Female patients aged 20-85 years
- Patients eligible for excision/lumpectomy
- Volume deficit compatible with an implant volume of 70 cc
- Adequate hematopoietic functions
- Good general health and mentally sound
- Confirmation of non-malignant lesion (B2 and B3) with no discordance between biopsy and radiological imaging
- Patients able and willing to give written informed consent form

Exclusion criteria

- Pregnant or breastfeeding women or women who have nursed a child three months within enrolment
- Non-malignant lesions (B2 and B3) with discordance between biopsy and radiological imaging
- Infection of the surgical site confirmed pre-operatively by clinical examination
- Acute or chronic severe renal insufficiency (creatinine values < 180 µmol/L)
- History of severe asthma or allergies
- Autoimmune disease
- Subject with actual concomitant malignancies, lobular neoplasm, metastatic breast carcinoma, sarcoma, malignant phyllodes lesions, or Paget's disease
- Subject who are known to be carriers of BCRA mutation
- Inability to undergo MRI
- Systemic infections in active phase
- Immunocompromised patients (HIV)
- Subjects who have participated in another study within the past 3 months
- Patients who received immunosuppressant therapy in the last 3 months
- Non-collaborative patients (severe physical disabilities or psychiatric disorders, as per specialist opinion).

Study Flowchart

	V-1 (Enrollement)	V0 (Surgery)	V1 (1 week)	V2 (2 weeks)	V3 (1 month)	V4 (3 months)	V5 (6 months)
Informed consent	X						
Inclusion/exclusion criteria	X						
Demographic data, medial history, weight	X						
Concomitant medications	X	X	X	X	X	X	X
Vital signs (BP,HR)	X	X					
ECG	X						
Physical examination	X	X	X	X	X	X	X
Breast lesion evaluation	X						
Biopsy (1)	X						
Blood sampling	X		X(b)				
Serum pregnancy test	X						
Patient diary	X	X	X	X	X	X	X
Ultrasound	X		X	X	X	X	X
Photos and chest measurements	X		X			X	X
Contrast MRI (2)	X						X
Surgery		X					
REGENERA implant		X					
Investigator's satisfaction		X(a)					X (c)
Patient's pain	X	X	X	X	X	X	X
BREAT-Q questionnaire	X				X		X
AEs assessment	X	X	X	X	X	X	X

(1) Previous biopsy accepted if performed within 6 months before the informed consent signature
(2) Previous MRI accepted if performed within 3 months before the informed consent signature
(a) On the implanting procedure, with a VAS scale, and an ad-hoc questionnaire to evaluate the performance of the device
(b) Evaluation only of complete blood count with differential; in case of infection or inflammation, exudate antibiogram is foreseen
(c) To evaluate overall satisfaction of surgical procedure and clinical outcome of patients, with a VAS scale

Fig. 1 Inclusion/exclusion criteria and study flowchart

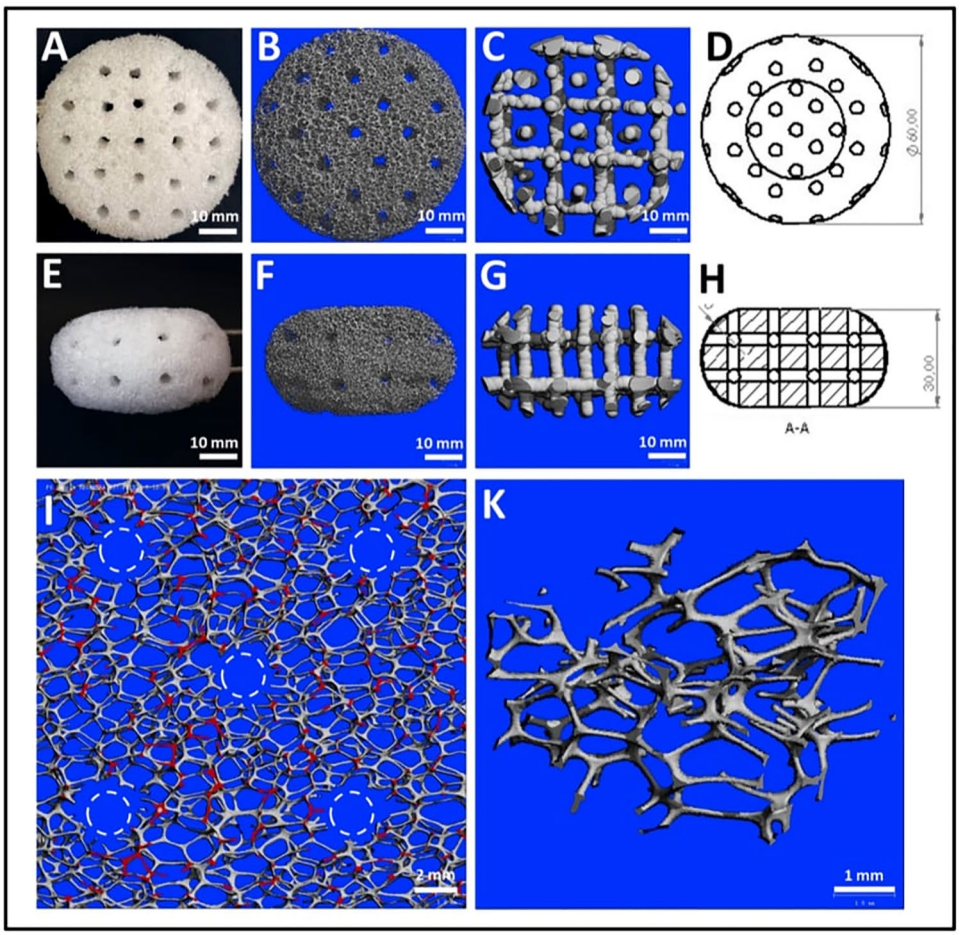


Fig. 2 Shape and geometry of the device. **A–D**: Dimensions are: 70 cc (±20 cc) volume, 60 (±5) mm diameter and 30 (±2) mm height; **E–H**: Top and lateral view of the device by means of photographic image, uCT scan-external view, uCT scan 3D-rendering of the channels network propagating through the device and graphical 3D visualization by SolidWorks, respectively; **I**: uCT scan micrograph showing the interconnection between channels and the porous structure of the matrix. The dashed white circles show the channel voids; **K**: high-magnitude uCT scan micrograph showing the open-pore structure of the device matrix. Scale bars are shown in the images

Contact: tissue/ bone; Contact duration: C-Long term (> 30 d)] and U.S. FDA recommendations.

Study flowchart and surgical technique

Each patient signed a written informed consent and performed 7 study visits, according to the study flowchart (Fig. 1). On the day of surgery, they received a single dose of antibiotic (i.e., first generation cephalosporin), were positioned supine on the table, with the arms stretched out in line with the shoulder, and underwent general anesthesia. After performing the lumpectomy and sending the surgical specimen for definitive histological examination, the surgeon prepared the device for implantation. The scaffold was removed from its outer package and remaining inside the inner package, was hydrated by 100 mL of physiologic solution for 10 min (±5 min). Finally, it was extracted from the internal bag and the excess liquid drops were dripped for 10 s taking care not to change its shape by over-handling. The scaffold was then placed in the cavity and covered with the surrounding tissue in a layered suture technique. A fall drainage was positioned at surgeon's discretion, and the skin was closed with an intradermal suture/ detached nylon stitches. The patient was discharged the same day.

Study endpoints and data analysis

The primary endpoint was the safety profile of the scaffold. The secondary endpoints were safety and feasibility of the implant procedure and performance of the device. The following research questions were addressed by the tools listed below:

Q1. "Are the device safe in humans and the implant procedure safe and feasible?" We assessed rate, type, and severity of all the adverse events (AEs) detected. Any new event not present at baseline, or worsening from baseline, reported by the patient, the investigator, or the medical records was considered an AE.

Q2. Device performance:

Q2a. "How effectively does it replace the removed tissue?" Three independent investigators evaluated breast appearance before (V-1) and after surgery (at V1, V4, and V5) by physical examination, standardized photographs (Fig. 3) and anthropomorphic measurements (Fig. 4). Ptosis [14], nipple position, areola and breast size for

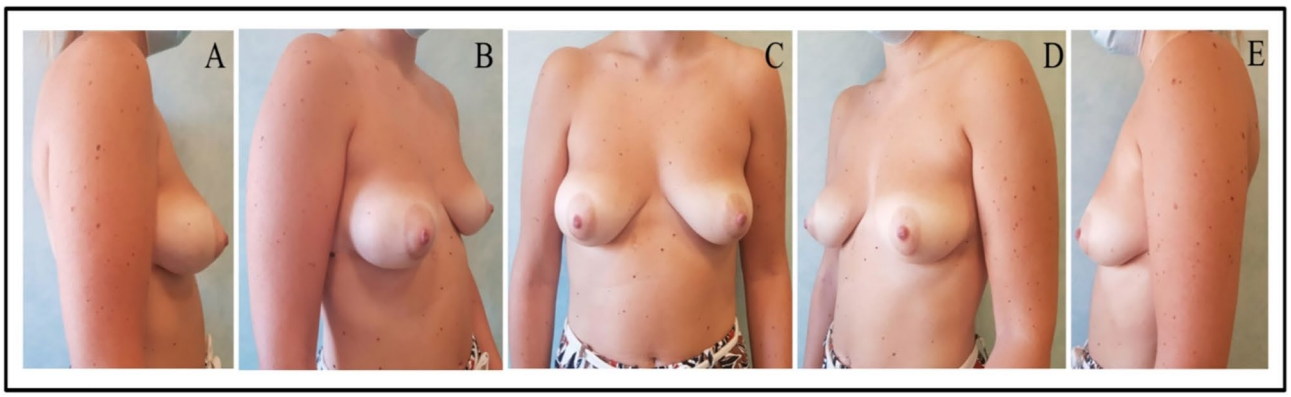


Fig. 3 Standardized upright photographs of the breast: **A.** Right lateral; **B.** Right oblique; **C.** Anterior; **D.** Left oblique; **E.** Left lateral

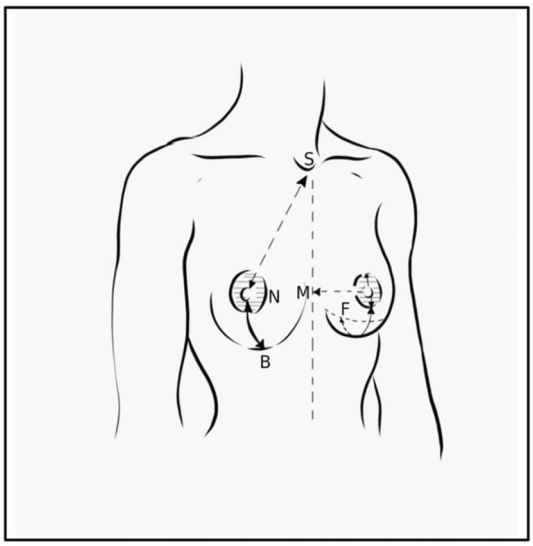


Fig. 4 Anthropomorphic measurements taken between labeled landmarks: sternal notch (S), nipple (N), sternum (M), breast base (B), inframammary fold (F)

each breast, and asymmetry of both breasts together were assessed. Post-operatively, an overall aesthetic score was also assigned using the Harvard scale [15]. Traditionally, this scale compares the two breasts, but we used it for the evaluation of the single breast over time, assessing whether and to what extent surgery had changed it.

Q2b. "Does it interfere with standard diagnostic imaging techniques?" Two experienced radiologists compared qualitatively transparency to ultrasound (US) and magnetic resonance imaging (MRI) between V-1 and V5 to identify some findings indicating the relationship with the surrounding tissues, and the resorption and repopulation processes. For some parameters, we also assigned a score to facilitate evaluation by different operators (Fig. 5).

Q2c."Does its use affect patient's QoL?" The BREAST-Q© questionnaire [16] was administered to patients at V-1, V3 and V5, quantifying the change of scores between V3 and V5 vs. baseline. Patient's pain was evaluated with a VAS scale from enrollment onwards.

Q2d. "Is it satisfactory from the investigator's point of view in terms of simplicity of the technique, surgical times, and overall results?" A VAS scale was administered to the surgeon at V0 and V5 to assess satisfaction for the implanting procedure and the final outcome, respectively.

Data are summarized by descriptive statistics: continuous variables are presented as mean value and standard deviation, categorical variables are summarized using counts of subjects and percentages.

Results

Baseline characteristics

The first 5 patients enrolled were included in this interim analysis. All were Caucasian, with mean age of 39 ± 11 years, and mean weight of 59.8 ± 12.8 kg. On imaging, the mean size of the lesion was 52 mm and biopsy identified 4 fibroadenoma and 1 non-proliferative dysplasia. All patients had a regular intraoperative and post-operative course during hospitalization, and were discharged the same day. The definitive histology showed 4 fibroadenoma, and 1 benign phyllodes with average size of 53.4 mm.

Safety analysis

Four patients (80%) had at least one AE. Overall, 17 AEs were reported and included: headache, dyspepsia, painful respiration, fatigue, discomfort on surgical incision, pruritus, breast or arm pain/discomfort. Eight AEs (47.0%) occurred within one week from surgery, 2 (11.8%) within two weeks,

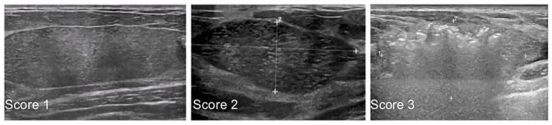
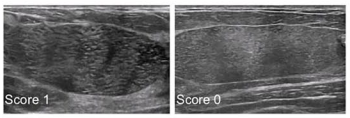
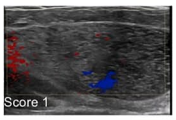
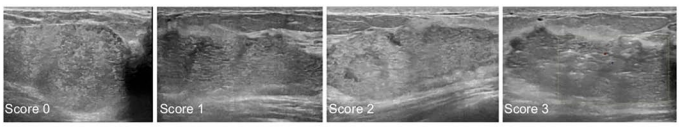
Finding evaluated	Description	Result of the evaluation	Score	Representative Images
Micro-artifacts	Heterogeneous US pattern, characterized by the presence of multiple and millimeter hypo and hyperechoic sectorial microartifacts	Homogeneity (absence of micro-artifacts)	0	
		Mild inhomogenicity	1	
		Moderate inomogenicity	2	
		Extreme heterogenicity (max. presence of micro-artifacts)	3	
Internal hypoechoic bands	Hypoechoic parallel bands (~1 cm spacing)	Present	1	
		Absent	0	
Color and Power Doppler findings	Peri- and intra-prosthetic vascularization	Present	1	
		Absent	0	
Periprosthetic hyperechogenicity	Hyperechoicity of the tissue placed in the immediate vicinity of the prosthesis	Absence of hyperechoicity	0	
		Mild hyperechoicity	1	
		Moderate hyperechoicity	2	
		Maximum hyperechogenicity	3	

Fig. 5 Ultrasound evaluation: Findings analyzed and score system used

4 (23.5%) within 1 month, 1 (5.9%) within 3 months, and 2 (11.8%) within 6 months. No AE occurred during surgery or had a possible relationship with the study product: 7 (41.2%) had no relationship and 10 (58.8%) were unlikely related. Concerning severity, none was serious, 5 (29.4%) were mild, 11 (64.7%) moderate, and only 1 (5.9%) severe, the latter consisting in arm pain. No alterations were never found on laboratory findings, vital signs, and physical examinations.

Performance analysis

Concerning breast appearance, the concordance between investigators was 100% pre and post-operatively. At both time-points, breast asymmetry was judged "absent" and "mild" in 2 and 3 patients, respectively. Since shape, size, ptosis and symmetry were unchanged with respect to baseline, and the scaffold was no longer detectable on palpation, the overall aesthetic score assigned was "Excellent" in 100% of cases (Fig. 6).

Under US (Fig. 7), in all patients at V1, the 6 cm oval-shaped scaffold had circumscribed or micro-lobulated margins, no shadow cones or posterior wall reinforcements, and an heterogeneous pattern characterized by multiple millimeter hypo and hyper-echoic micro-artifacts. In all patients peri and intra-scaffold doppler signals, hyperechogenicity of the surrounding tissue and internal millimeter hypo-echoic bands, parallel to each other and placed at a constant distance of 1 cm, were detected. Over time, the size of the scaffold remained unchanged or slightly reduced in 3 (60%) and 2 (40%) patients, respectively. Micro-artifacts, internal hypo-echoic bands and surrounding tissue hyperechogenicity reduced dimensionally and numerically in 100%, 80% (i.e.,4/5) and 40% (i.e., 2/5) of patients, respectively. In 3 patients (60%), an anechoic peri-scaffold collection, more evident at the poles was detected. No patient ever presented axillary lymphadenopathy.

At MRI (Fig. 7), in 2 patients, the scaffold had a reduced size. All had regular margins, hypo-intensity in T1-weighted sequences (T1ws) and hyper-intensity in T2-weighted sequences (T2ws). In 3 (60%) patients, along its edges, flat minus focal points corresponding to images of plus capturing contrast were detected in T2ws and after contrast medium injection, respectively. Always, it showed a rim enhancement.

Concerning the BREAST-Q©, at V-1 the mean of the four dimensions analyzed were: 52.2 ± 6.2, 71.4 ± 13.1, 28.4 ± 15.7 and 71.2 ± 20.6 for satisfaction with breast, psychosocial, physical and sexual well-being, respectively. At V3, all dimensions increased, with a mean value of 81.4 ± 11.0, 83.4 ± 12.2, 40.8 ± 14.6, and 82.8 ± 12.4, respectively. At V5, the dimensions physical and sexual well-being further increased with respect to baseline and V3: mean 42 ± 14.3 and 84.8 ± 20.8, respectively. Satisfaction with breast and psychosocial well-being slightly decreased from V3 but were both increased with respect to baseline: mean 76 ± 15.64 and 82 ± 15.12, respectively.

Pain maximum mean value was 2.2 ± 1.9 at V0, with maximum value of 5. At V1,V2 and V3, the mean values were 1.2 ± 1.1; $1 \pm 1,2$; and 0.2 ± 0.4, respectively. At V4 and V5, all patients reported a 0 score. Mean investigator's satisfaction at V0 and V5 was 8.6 ± 0.5 and 9.8 ± 0.4, respectively.

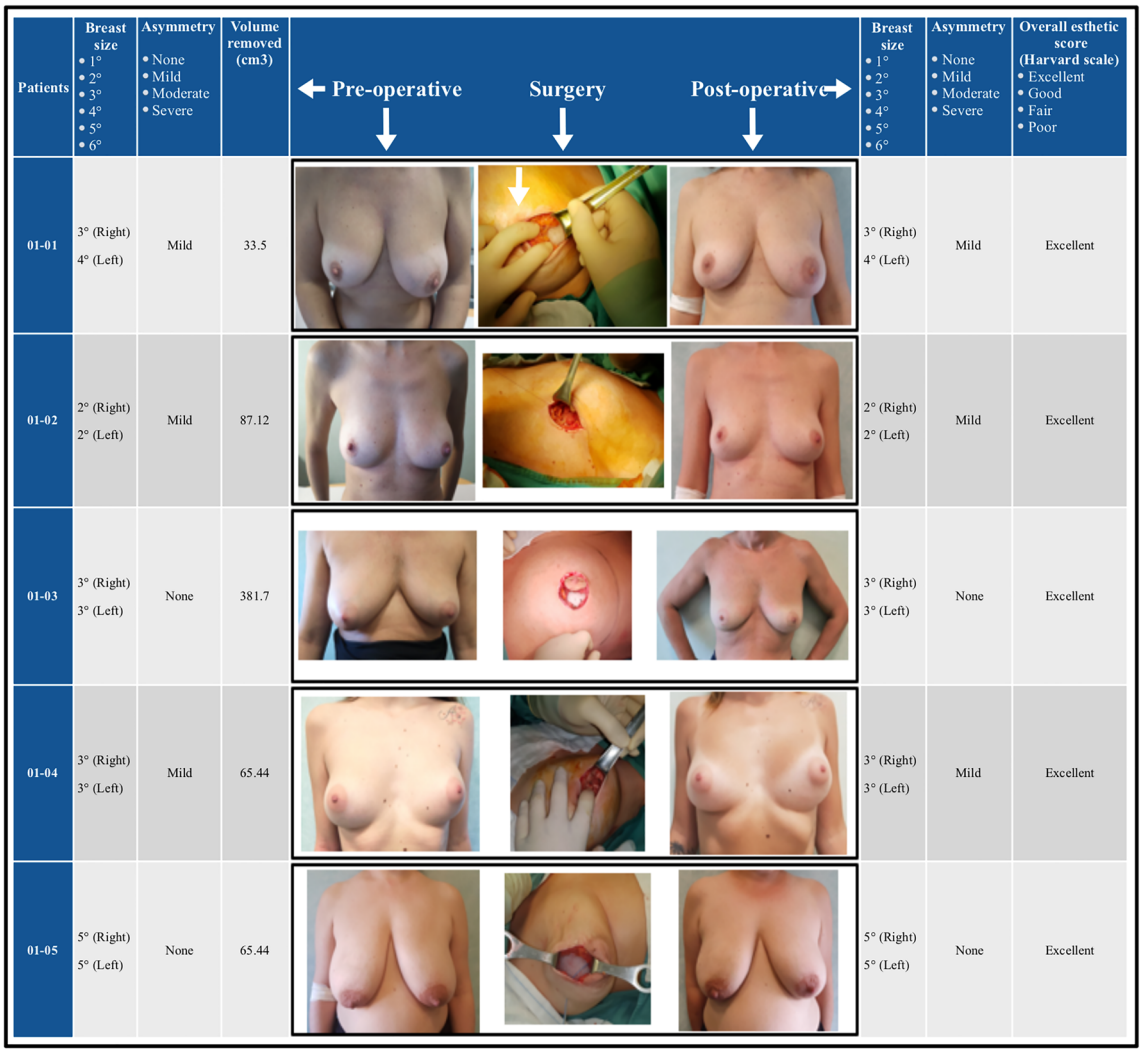


Fig. 6 Breast appearance: Breast size, degree of breast asymmetry and volume of tissue removed (cubic centimeters) in the pre-operative evaluation (V-1); breast size, degree of asymmetry and overall aesthetic score (Harvard Scale) in the final post-operative evaluation (V5)

Discussion

The rationale of this FIH pilot study lies in the fact that all the currently available options for optimizing the esthetic results after BCS have well-known limitations [7]. Therefore, a growing interest is addressed to the tissue engineering (TE) paradigm, which represents an attractive alternative [17–33].

Since the idea of a tissue engineered breast has been hypothesized, several biomaterials have been investigated to be used as implantable scaffold providing mechanical support and allowing regeneration of host tissue until it is mature enough to support itself [19–22]. These scaffolds, which must have biodegradability, low immunogenicity, and porous architecture, can regenerate the desired tissue in two main ways: either by scaffolds recruiting native cells to migrate and proliferate inside them (in vivo TE approach), or by pre-culturing cells into scaffolds before implantation (in vitro TE approach). The published literature reports a number of TE studies addressing diverse tissues, both in vivo and in vitro [19, 27–33], but translation from research to clinical reality still remains a challenge. FIH studies have successfully employed the TE approach to repair blood vessels, bladder, nasal nostril and vaginal vault [34–37], but as stated by Morrison et al. [18], these are tissues either thin enough to relay in nutrient diffusion until neovascularization occurs or with a low metabolic rate that allows them to survive under hypoxic conditions. By contrast, adipose tissue of the mammary gland poses a different challenge due to its thickness and metabolic requirements.

In this context, this FIH study consisting of an in vivo TE approach, can have remarkable impact on the research and clinical application of the TE paradigm to highly vascularized tissues. Indeed, the device used is a bioactive synthetic

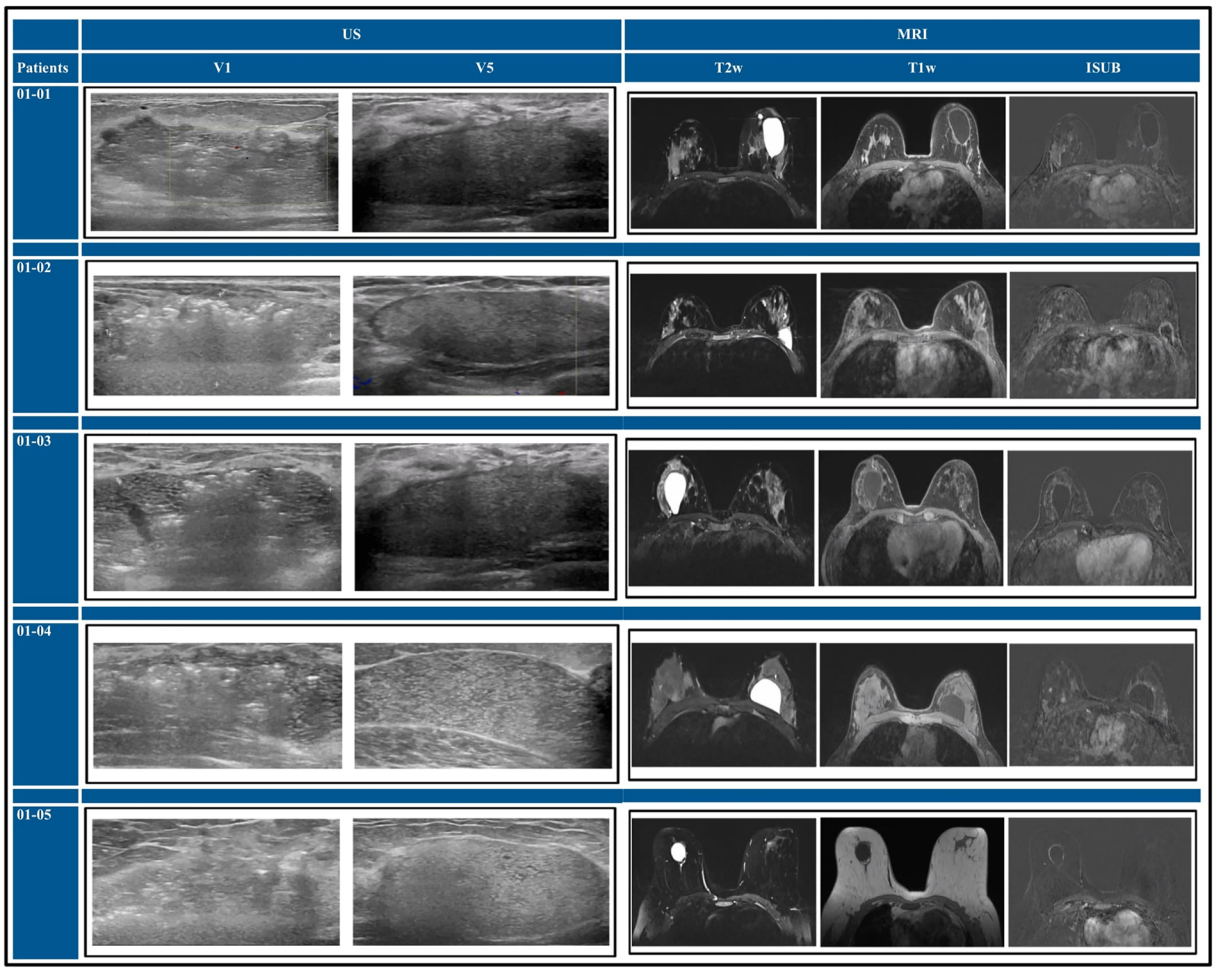


Fig. 7 US and MRI imaging: Findings at 1 week from surgery (V1) and after six months (V5)

scaffold intended for regenerating soft-tissue resembling fat. The aim is to restore a natural breast in terms of shape and consistency after BCS, thanks to its capacity to provide biological and mechanical support and to promote cell infiltration and vascular network development while being degraded and replaced by the patient's soft tissue. The preliminary data collected in this study are strongly supportive that the use of BCS combined with the unique structure of this scaffold, could achieve a twofold objective. First, to improve patient's QoL, by means of a minimally invasive surgical technique. Second, to reduce health-related costs, thanks to the fact that this is a simple, unilateral and rapid procedure, requiring shorter times of occupancy of the operating room, hospitalization and recovery. Indeed, surgery simply consists of dissecting the lesion and implanting the device, filling the volume deficit with a fully accessible scaffold for tissue ingrowth. Given its characteristics, and based on the pre-clinical studies, the only AE with moderate possibility of occurrence using this device, is inflammation [38]. Indeed, the biomaterial, it is made of, belongs to the family of synthetic polymers, more specifically to the cross-linked aliphatic poly(urethane-ester-ether)s group. Crosslinked porous polyurethane-based matrices are already used in the clinical setting for wound healing and other indications [39] and the decision to use aliphatic in lieu of aromatic polyisocyanates was made to ensure the highest device safety, especially in terms of biocompatibility of the degradation subproducts. Besides this remarkable biocompatibility, the main advantage of this biomaterial is that its macromolecular structure as well as physicochemical and mechanical properties can be finely tuned to closely match those of human adipose tissue, making it particularly suitable for the use for which it was tested in this study.

In terms of safety, the preliminary data collected show that the device and procedure are safe in humans. Indeed, laboratory tests, vital signs and physical examination never presented clinically relevant alterations after the implantation of the scaffold, and a limited number of AEs was observed, with some noteworthy aspects. First, the timing: the majority (82.3%) occurred within the first month and

47% of these within the first week. Therefore, since the normal recovery times after lumpectomy is around 30 days, the device implanted does not seem to impact on them. Second, the relationship with the device: 41.2% were not related at all (i.e., headache, dyspepsia, fatigue), 58.8% were more likely related to surgery itself (i.e., breast pain) and none had a relationship with the device. Third, the severity: just 1 (5.9%) was classified by the patient as 'severe' and consisted of arm pain, which is a symptom more likely related to the position during surgery or to posture defects rather than to the device implanted, and anyway resolved within the study observation without any therapy.

The device performance was analyzed by different tools.

To assess how effectively it replaced the removed tissue, not changing breast appearance, we used an aesthetic evaluation. To minimize subjectivity, we combined photographic evaluation and physical examination with anthropometric measurements, and used a validated evaluation scale. In all patients, after 6 months, the surgical scar was in order, the scaffold was no longer detectable on palpation, and breast shape, size, ptosis and symmetry were unchanged with respect to the pre-operative evaluation with an "Excellent" overall aesthetic score. These data suggest that the device does not impair wound healing and does not change breast appearance.

Given that in these first 5 patients the average size of the breasts was a III° measure with an average size of the removed lesions of 50 mm, future objectives will include evaluating the impact of the device on the aesthetic outcome in case of lesions larger in relation to the breast volume.

A qualitative evaluation of transparency to US and MRI was used to assess potential interference with imaging with a twofold objective: to understand whether the scaffold affected the ability to study the surrounding tissue, and to identify findings indicating the relationship with the surrounding tissues and evidences of degradation, neovascularization and tissue regeneration. Data suggest that the device does not impact on the first aspect. Regarding the second objective, the internal micro-artifacts observed at US in all patients at V1, were attributed to its structure and to liquids and gases due to surgery. Accordingly, their progressive reduction was interpreted as degradation/repopulation of the scaffold and/or reabsorption of liquids and gases. The internal hypo-echoic bands, always detected at V1 and reduced in 80% of patients, were attributed to the channels of the scaffold even if it isn't clear why they reduced only in some cases. The constant presence of peri and intra-scaffold doppler signals, indicated neovascularization. The surrounding tissue hyperechogenicity always present at V1, and reduced in 40% of patients, was interpreted as edema undergoing resolution. This finding, as well as the minimal anechoic peri-scaffold collection attributed to reactive effusion (observed in 60% of patients) and the absence of lymphadenopathy, suggested absence of significant inflammation. At MRI, the hyperintensity in T2ws and the hypo-intensity in T1ws, supported the hypothesis of the presence of cellular vascularized tissue. The images of focal minuses along the edges of the device, corresponding to plus images capturing contrast detected in 60% of patients, were attributed either to fibrous pseudocapsule, compression of surrounding vessels, or enhanced angiogenesis. Finally, we observed that both at US and MRI, the scaffold maintained or slightly reduced its size, suggesting that the degradation speed is influenced by patient's related factors, whose nature will be clarified with a longer follow-up.

To assess patient's perception of aesthetic and functional outcomes, post-operative pain and impact on QoL were analyzed. The mean maximum pain value was $2.2 + -1.9$ at V0 with some noteworthy elements: it was always very low, and completely absent after 3 months. Concerning QoL, all dimensions analyzed increased at V3 vs V-1. At V5, two dimensions (physical and sexual well-being) further increased vs baseline and V3 (mean 42 ± 14.3 and 84.8 ± 20.8, respectively), while satisfaction with breast and psychosocial well-being slightly decreased from V3 but were both increased vs baseline (mean 76 ± 15.64 and 82 ± 15.12, respectively). Overall, these data suggest that this surgical technique is minimally invasive, with low post-operative pain and positive impact on patient's QoL.

Scores of investigator's satisfaction with the implanting procedure (at V0) and overall results (at V5) were high at both time-points (mean value 8.6 ± 0.5 and 9.8 ± 0.4, respectively).

Therefore, albeit on a very limited number of patients treated, we observed a positive outcome both in terms of safety and performance.

Our study has some limitations.

First, the small sample size and short follow-up. Both were deemed adequate for the sole purpose of acquiring preliminary information on an innovative device and surgical technique to design an adequate development plan and to understand if the study could continue or should be stopped. The data reported herein, are the preliminary results of the interim analysis on the first 5 patients enrolled, so they will be integrated with the final results on the entire patient population. Furthermore, an extension study (5-year follow-up) has already been undertaken on the same sample, and a multi-center study with larger sample and longer follow-up has gathered favorable opinion from local hospital Ethical Committees (among them, Azienda Ospedaliero-Universitaria Pisana; AOUP) and is awaiting approval from the National Competent Authorities.. Therefore, only future studies, also of a comparative nature with consolidated techniques, will give us more certainty on the reliability of this new surgical approach.

Second, no data on the impact of the device on mammography are yet available at 6-month follow-up, because mammography must be performed annually. These data will be provided by the extension study.

Third, the choice of the imaging findings to be analyzed and their interpretation. Both may change in the future due to a better understanding of the modifications occurring inside and around the scaffold.

To conclude, the possibility of using TE in breast reconstruction has enormous potential in terms of invasiveness, cosmetic results, impact on patient's QoL and healthcare costs.

Data obtained from the interim analysis on the first 5 patients enrolled in this study suggest that the surgical technique used is safe and highly performing. None of the AEs recorded was device-related, the scaffold did not interfere with US and MRI ability to accurately study the surrounding tissue, and the aesthetic outcome as well as the patient's and surgeon's satisfaction were excellent. Nevertheless, since these data are preliminary, analysis of data relating to the entire study population and further investigations with greater follow-up and on a larger sample are mandatory to draw more reliable conclusions.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MDM, BF, IG, MT, AT, FM, MG, and MR. The first draft of the manuscript was written by MDM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data availability The data presented in this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare the following financial/non-financial interests which may be considered as potential competing interests: Authors I.G., M.T., A.T. and F.M. are share-holders and members of the board of directors of Tensive S.r.l. They have received the grant H2020-EIC-SMEInst-2018–2020 (Grant agreement number: 812002) from the European Commission. The remaining authors have no relevant financial or non-financial interests to disclose.

Ethical approval This study involving human participants, was conducted in accordance with the ethical standards of the institutional (AOUP Ethical Committee-Protocol # 51965, 11 October 2018) and national (Italian Ministry of Health-0066926-05/12/2018-DGDMF-MDS-P) research committee and with the 1964 Helsinki declaration and its later amendments and the Good Clinical Practice principles.

Informed consent Written informed consent was obtained from all individual participants included in the study.

References

1. American Cancer Society (2021). How common is breast cancer?, https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html; Accessed 15 Oct 2022
2. Ojala K, Meretoja TJ, Leidenius MH. Aesthetic and functional outcome after breast conserving surgery - Comparison between conventional and oncoplastic resection. Eur J Surg Oncol. 2017;43(4):658–64. https://doi.org/10.1016/j.ejso.2016.11.019.
3. Van Dongen JA, Voogd AC, Fentiman IS, Legrand C, Sylvester RJ, Tong D, et al. Long-term results of a randomized trial comparing breast-conserving therapy with mastectomy: European Organization for Research and Treatment of Cancer 10801 trial. J Natl Cancer Inst. 2000;92(14):1143–50. https://doi.org/10.1093/jnci/92.14.1143.
4. Franceschini G, Martin Sanchez A, Di Leone A, Magno S, Moschella F, Accetta C, et al. New trends in breast cancer surgery: a therapeutic approach increasingly efficacy and respectful of the patient. G Chir. 2015;36(4):145–52. https://doi.org/10.11138/gchir/2015.36.4.145.
5. Veronesi U, Cascinelli N, Mariani L, Greco M, Saccozzi R, Luini A, et al. Twenty-year follow-up of a randomized study comparing breast-conserving surgery with radical mastectomy for early breast cancer. N Engl J Med. 2002;347(16):1227–32. https://doi.org/10.1056/NEJMoa020989.
6. Vidya R, Leff DR, Green M, McIntosh SA, St John E, Kirwan CC, et al. Innovations for the future of breast surgery. Br J Surg. 2021;108(8):908–16. https://doi.org/10.1093/bjs/znab147.
7. Behluli I, Le Renard PE, Rozwag K, Oppelt P, Kaufmann A, Schneider A. Oncoplastic breast surgery versus conventional breast-conserving surgery: a comparative retrospective study. ANZ J Surg. 2019;89(10):1236–41. https://doi.org/10.1111/ans.15245.
8. Tocchio A, Tamplenizza M, Martello F, Gerges I, Rossi E, Argentiere S, et al. Versatile fabrication of vascularizable scaffolds for large tissue engineering in bioreactor. Biomaterials. 2015;45:124–31. https://doi.org/10.1016/j.biomaterials.2014.12.031.
9. Tamplenizza M, Tocchio A, Gerges I, Martello F, Martelli C, Ottobrini L, et al. In Vivo Imaging Study of Angiogenesis in a Channelized Porous Scaffold. Mol Imaging. 2015. https://doi.org/10.2310/7290.2015.00011.
10. Alkhouli N, Mansfield J, Green E, Bell J, Knight B, Liversedge N, et al. The mechanical properties of human adipose tissues and their relationships to the structure and composition of the extracellular matrix. Am J Physiol Endocrinol Metab. 2013;305(12):E1427–35. https://doi.org/10.1152/ajpendo.00111.2013.
11. Rossi E, Gerges I, Tocchio A, Tamplenizza M, Aprile P, Recordati C, et al. Biologically and mechanically driven design of an RGD-mimetic macroporous foam for adipose tissue engineering applications. Biomaterials. 2016;104:65–77. https://doi.org/10.1016/j.biomaterials.2016.07.004.
12. Young DA, Choi YS, Engler AJ, Christman KL. Stimulation of adipogenesis of adult adipose-derived stem cells using substrates that mimic the stiffness of adipose tissue. Biomaterials. 2013;34(34):8581–8. https://doi.org/10.1016/j.biomaterials.2013.07.103.
13. Gerges I, Tamplenizza M, Martello F, Recordati C, Martelli C, Ottobrini L, et al. Exploring the potential of polyurethane-based soft foam as cell-free scaffold for soft tissue regeneration. Acta

Biomater. 2018;73:141–53. https://doi.org/10.1016/j.actbio.2018.04.011.
14. Reginault P. Breast ptosis. Definition and treatment. Clin Plast Surg. 1976;3(2):193–203 (**PMID: 1261176**).
15. Cardoso MJ, Cardoso JS, Vrieling C, Macmillan D, Rainsbury D, Heil J, et al. Recommendations for the aesthetic evaluation of breast cancer conservative treatment. Breast Cancer Res Treat. 2012;135(3):629–37. https://doi.org/10.1007/s10549-012-1978-8.
16. Pusic AL, Klassen AF, Scott AM, Klok JA, Cordeiro PG, Cano SJ. Development of a new patient-reported outcome measure for breast surgery: the BREAST-Q. Plast Reconstr Surg. 2009;124(2):345–53. https://doi.org/10.1097/PRS.0b013e3181aee807.
17. Huss FR, Kratz G. Mammary epithelial cell and adipocyte co-culture in a 3-D matrix: the first step towards tissue-engineered human breast tissue. Cells Tissues Organs. 2001;169(4):361–7. https://doi.org/10.1159/000047903.
18. Morrison WA, Marre D, Grinsell D, Batty A, Trost N, O'Connor AJ. Creation of a Large Adipose Tissue Construct in Humans Using a Tissue-engineering Chamber: A Step Forward in the Clinical Application of Soft Tissue Engineering. EBioMedicine. 2016;6:238–45. https://doi.org/10.1016/j.ebiom.2016.03.032.
19. Wang X, Reagan MR, Kaplan DL. Synthetic adipose tissue models for studying mammary gland development and breast tissue engineering. J Mammary Gland Biol Neoplasia. 2010;15(3):365–76. https://doi.org/10.1007/s10911-010-9192-y.
20. Debels H. Advances in tissue engineering; a novel technology making use of an in vivo vascularized chamber. Acta Chir Belg. 2015;115(2):104–10. https://doi.org/10.1080/00015458.2015.11681078.
21. Chhaya MP, Melchels FP, Holzapfel BM, Baldwin JG, Hutmacher DW. Sustained regeneration of high-volume adipose tissue for breast reconstruction using computer aided design and biomanufacturing. Biomaterials. 2015;52:551–60. https://doi.org/10.1016/j.biomaterials.2015.01.025.
22. Chhaya MP, Balmayor ER, Hutmacher DW, Schantz JT. Transformation of Breast Reconstruction via Additive Biomanufacturing. Sci Rep. 2016;6:28030. https://doi.org/10.1038/srep28030.
23. Findlay MW, Dolderer JH, Trost N, Craft RO, Cao Y, Cooper-White J, et al. Tissue-engineered breast reconstruction: bridging the gap toward large-volume tissue engineering in humans. Plast Reconstr Surg. 2011;128(6):1206–15. https://doi.org/10.1097/PRS.0b013e318230c5b2.
24. Aimei J, Ming L, Wenjing D, Yilong D, Yanmei W. Improvement of the survival of human autologous fat transplantation by adipose-derived stem-cells-assisted lipotransfer combined with bFGF. Scie World J. 2015;2015:968057. https://doi.org/10.1155/2015/968057.
25. Wang L, Johnson JA, Zhang Q, Beahm EK. Combining decellularized human adipose tissue extracellular matrix and adipose-derived stem cells for adipose tissue engineering. Acta Biomater. 2013;9(11):8921–31. https://doi.org/10.1016/j.actbio.2013.06.035.
26. Cho SW, Song KW, Rhie JW, Park MH, Choi CY, Kim BS. Engineered adipose tissue formation enhanced by basic fibroblast growth factor and a mechanically stable environment. Cell Transplant. 2007;16(4):421–34. https://doi.org/10.3727/000000007783464795.
27. Cho SW, Kim SS, Rhie JW, Cho HM, Choi CY, Kim BS. Engineering of volume-stable adipose tissues. Biomaterials. 2005;26(17):3577–85. https://doi.org/10.1016/j.biomaterials.2004.09.013.
28. Rossi E, Guerrero J, Aprile P, Tocchio A, Kappos EA, Gerges I, et al. Decoration of RGD- mimetic porous scaffolds with engineered and devitalized extracellular matrix for adipose tissue regeneration. Acta Biomater. 2018;73:154–66. https://doi.org/10.1016/j.actbio.2018.04.039.
29. Xiao X, Zhou S, Wan J, Dong Z, Wang Y, Feng C. Pre-shaped large-volume engineered vascularized pedicled adipose flaps in a rabbit model: a two stage tissue engineering chamber-based procedure. J Biomater Tissue Eng. 2017;7:261–8. https://doi.org/10.1166/JBT.2017.1569.
30. Gentile P, Orlandi A, Scioli MG, Di Pasquali C, Bocchini I, Curcio CB, et al. A comparative translational study: The combined use of enhanced stromal vascular fraction and platelet-rich plasma improves fat grafting maintenance in breast reconstruction. Stem Cells Transl Med. 2012;1(4):341–51. https://doi.org/10.5966/sctm.2011-0065.
31. Tsuji W, Inamoto T, Ito R, Morimoto N, Tabata Y, Toi M. Simple and longstanding adipose tissue engineering in rabbits. J Artif Organs. 2013;16(1):110–4. https://doi.org/10.1007/s10047-012-0670-4.
32. Yao R, Zhang R, Lin F, Luan J. Biomimetic injectable HUVEC-adipocytes/collagen/alginate microsphere co-cultures for adipose tissue engineering. Biotechnol Bioeng. 2013;110(5):1430–43. https://doi.org/10.1002/bit.24784.
33. Rehnke RD, Schusterman MA 2nd, Clarke JM, Price BC, Waheed U, Debski RE, et al. Breast reconstruction using a three-dimensional absorbable mesh scaffold and autologous fat grafting: a composite strategy based on tissue-engineering principles. Plast Reconstr Surg. 2020;146(4):409e–13e. https://doi.org/10.1097/PRS.0000000000007172.
34. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet. 2006;367(9518):1241–6. https://doi.org/10.1016/S0140-6736(06)68438-9.
35. Fulco I, Miot S, Haug MD, Barbero A, Wixmerten A, Feliciano S, et al. Engineered autologous cartilage tissue for nasal reconstruction after tumour resection: an observational first-in-human trial. Lancet. 2014;384(9940):337–46. https://doi.org/10.1016/S0140-6736(14)60544-4.
36. Olausson M, Patil PB, Kuna VK, Chougule P, Hernandez N, Methe K, et al. Transplantation of an allogeneic vein bioengineered with autologous stem cells: a proof-of-concept study. Lancet. 2012;380(9838):230–7. https://doi.org/10.1016/S0140-6736(12)60633-3.
37. Raya-Rivera AM, Esquiliano D, Fierro-Pastrana R, López-Bayghen E, Valencia P, Ordorica-Flores R, et al. Tissue-engineered autologous vaginal organs in patients: a pilot cohort study. Lancet. 2014;384(9940):329–36. https://doi.org/10.1016/S0140-6736(14)60542-0.
38. Gerges I, Tamplenizza M, Martello F, Koman S, Chincarini C, Recordati C, et al. Conditioning the microenvironment for soft tissue regeneration in a cell free scaffold. Sci Rep. 2021;11:13310. https://doi.org/10.1038/s41598-021-92732-9.
39. Bulgheroni P, Bulgheroni E, Regazzola G, Mazzola C. Polyurethane scaffold for the treatment of partial meniscal tears Clinical results with a minimum two-year follow-up. Joints. 2014;1(4):161–6. https://doi.org/10.11138/jts/2013.1.4.161.

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