Additive manufacturing and technical strategies for improving outcomes in breast reconstructive surgery

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ABSTRACT

It has been widely reported that breast reconstruction improves the quality of life of women who undergo mastectomy for breast cancer. This approach provides many psychological advantages. Today, different techniques are available for the breast oncoplastic surgeon that involve the use of breast implants and autologous tissues, also offering interesting results in terms of aesthetic and patient-reported outcomes. On the other hand, advanced technologies and design strategies (i.e. design for additive manufacturing, reverse engineering) may allow the development of customised porous structures with tailored morphological, mechanical, biological, and mass transport properties. For this reason, the current study deals with the challenges, principles, and methods of developing 3D additive manufactured structures in breast reconstructive surgery. Specifically, the aim was to design 3D additive manufactured poly(ε-caprolactone) scaffolds with different architectures (i.e. lay-down patterns). Preliminary mechanical and biological analyses have shown the effect of the lay-down pattern on the performances of the manufactured structures.

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**Keywords:** additive manufacturing; breast reconstructive surgery; fat grafting; reverse engineering; scaffold design; pore geometry and lay-down pattern; mechanical and functional properties

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1. Introduction

Breast reconstruction provides a significant improvement in the quality of life of women undergoing mastectomy for breast cancer [1].

Immediate reconstruction does not negatively impact on oncological outcomes, does not delay the commencement of adjuvant therapies, and does not impair postoperative oncological surveillance [2], [3].

The conservative mastectomies (nipple areola complex (NAC)-sparing, skin-sparing and skin-reducing) allow the complete preservation of the breast envelope in patients, without involvement of the skin or the NAC [4]-[6].

Currently, breast surgeons can choose among many surgical techniques for breast reconstruction, with the opportunity of offering women the best results in terms of aesthetic results and postoperative quality of life, using both silicone breast implants and autologous tissues [7].

The patient’s wishes must drive every choice in the decision-making for breast reconstruction, aiming at tailored surgical treatments [8], [9].

Implant-based reconstructive options are widely used, even though they could be associated with postoperative complications and with a worsening of the aesthetic results over time [7]-[10].

Capsular contracture represents the most common complication. Other complications have also been described in association with breast implants, such as ruptures, infections, late seromas, and the development of an extremely rare form of lymphoma, the breast implant-associated anaplastic large cell lymphoma (BIA-ALCL), [11].

In particular, the possible development of BIA-ALCL has recently raised some doubts about the safety of silicone breast implants.

Researchers are searching for alternatives to implants for breast reconstruction.

A possible alternative to breast implants is represented by myocutaneous pedicled and muscle-sparing free flaps, already used by several reconstructive surgeons with good outcomes.

However, breast reconstruction with autologous tissue flaps is not exempt from complications and reinterventions. Moreover, the autologous tissue-based surgical procedures are time consuming and expensive when compared to implant-based reconstruction.

The Mastectomy Reconstruction Outcomes Consortium (MROC) Study, a prospective multi-centre trial, recruited patients undergoing breast reconstruction (implant-based and autologous-tissue-based) following mastectomy from 11 centres across North America from February 2012 to July 2015 [12], [13].

The MROC study reported two-year complications rate following autologous tissue-based reconstruction of 47 % versus 26.6 % with implant-based techniques and reoperation rates of 27.4 % with autologous tissues versus 15.5 % with implants.

These data must be taken into account when considering autologous flaps as the first possible alternative to silicone implants for breast reconstruction.

We developed a new reconstructive technique with the aim of reducing surgical aggressiveness, improving cosmetic outcomes, and achieving long-lasting results, involving tissue regeneration supported by three-dimensional (3D) additively manufactured scaffolds with controlled morphology.

This method could represent a further evolution of the ‘hybrid reconstructive’ option that we have already presented, with the combined use of silicone implants and autologous fat tissue transplantation [14], [15].

3D-printed bioresorbable scaffolds will be positioned subcutaneously following NAC-sparing or skin-sparing mastectomies and filled with autologous fat tissue over 2–3 sessions to achieve a natural-shaped breast mound with a soft consistency and long-lasting aesthetic results.

This reconstructive option could also be offered to women undergoing postmastectomy radiotherapy (PMRT), representing a significant advantage when compared with silicone implants in the radiotherapy setting [16].

Future clinical applications of the 3D-engineered breast reconstruction will validate this innovative technique that will probably become a standard for the next generation breast surgeon.

In this scenario, the development of innovative breast devices would involve the reverse engineering approach and additively manufactured scaffolds combined with autologous fat grafting.

Thus, 3D scaffolds with autologous adipose-derived stem cells have been proposed for breast tissue engineering, as the strategy should be to appropriately place the device and to fill it with autologous fat tissue over a few sessions.

The customised porous structure filled with autologous fat tissue should be capable of maintaining the breast shape, as well as its natural consistency.

Further technical improvements (i.e. stromal vascular fraction derived growth factors) will be also taken into consideration.

For this reason, an integration of the basic concepts of regenerative medicine with advanced technologies (i.e. additive manufacturing), image capture, and analysis techniques should potentially lead to the design of 3D scaffolds with tailored architectural features and properties for breast tissue regeneration.

In brief, 3D virtual models of the breast may be generated starting from medical scans (i.e. computed tomography (CT), magnetic resonance imaging (MRI)) and 3D porous structures can be fabricated by fused deposition modelling (FDM/3D) fibre deposition, layer-by-layer, according to specific lay-down patterns.

In this context, to design innovative systems, the research may clearly benefit from the development of advanced technologies [17] and methodologies of analysis [18]-[21] in different fields, as well as from the engineering of biomaterials [22], [23] and 3D porous structures with enhanced and tailored properties for tissue regeneration [24], [25].

Accordingly, the aim of the current study was to design 3D additively manufactured poly(ε-caprolactone) scaffolds with different architectures (i.e. lay-down patterns).

Preliminary mechanical and biological analyses were carried out to analyse the effect of the lay-down pattern on the performances of the manufactured structures.

1. materials and methods

Poly(ε-caprolactone) (PCL, CAPA 6500, *M*w = 50,000) pellets were employed and 3D scaffolds (length *L* of 7.0 mm, width *W* of 7.0 mm, height *H* of 8.0 mm) were fabricated by FDM/3D fibre deposition technique, using an extrusion-based system.

The pellets were heated to 100°C and the 3D scaffolds were built layer by layer, as the molten PCL was extruded/injected through a needle with an inner diameter of 300 µm.

The PCL filaments were deposited according to three sequences of stacking (i.e. lay-down patterns) (0/90°, 0/60/120° and 0/45/90/135°).

A filament distance and a slice thickness of 650 and 280 µm were employed, respectively. Further process parameters were: extrusion pressure of 5 bar, deposition speed of 10 mm/s, screw velocity of 30 rpm.

Three different lay-down patterns were adopted that maintained a constant filament distance (i.e. centre-to-centre distance) of 650 μm. Thus, different pore geometries were obtained.

Mechanical compression tests were carried out on the manufactured scaffolds at a cross-head speed of 1 mm/min, up to a strain of 0.4 mm/mm, using an INSTRON 5566 testing machine.

The apparent stress (σ) and strain (ε) were calculated as reported below [25], [26]:

|  |  |
| --- | --- |
|  | (1) |
|  | (2) |

with F being the measured force, whereas ∆H represents the height variation of the PCL scaffold.

The slope of the initial linear portion of the stress–strain curve was considered to determine the compressive modulus.

The biological performances of the manufactured scaffolds were evaluated to analyse the effect of the lay-down patterns.

In brief, the PCL scaffolds were prepared for cell seeding following a protocol that has already been reported in the literature [25], [26]. The PCL scaffolds were seeded with adipose-derived mesenchymal stem cells (AMSCs) using 1 × 104 cells/sample.

The cell viability was evaluated at different time points using the Alamar Blue assay (AbD Serotec Ltd,UK).

Confocal laser scanning microscopy (CLSM) and rhodamine phalloidin staining were employed to analyse the cell adhesion and spreading at different time points.

The Image J software and a shape factor were used to analyse the CLSM images of the cell-scaffold constructs [25], [26].

The shape factor was calculated as follows:

|  |  |
| --- | --- |
|  | (3) |

with *P* and *A* representing the perimeter and the area of a cell, respectively.

As frequently reported, circular objects have the greatest area-to-perimeter ratio, and a shape factor of 1 represents a perfect circle. Thin thread-like objects are characterised by the lowest shape factor, which approaches zero [25], [26].

All the data were analysed by ANOVA, followed by the Bonferroni post hoc test.

Statistically significant values were defined as *p* < 0.05.

1. Results

The gel-filled breast implants generally consisted of a shell made of an elastomeric material (i.e., polydimethylsiloxane (PDMS) and a gel-like core (i.e. PDMS with a lower cross-linking degree).

With regard to the development of novel breast devices, the use of advanced technologies and the increasing knowledge of the structure–property relationship of the materials have led to the possibility of reproducing the complex viscoelastic properties, as well as the nonlinear and large-deformation behaviour.

Several strategies have been proposed for breast tissue repair/reconstruction and regeneration, also involving the combination of additive manufacturing techniques with an appropriate selection of materials that are already used for tissue engineering and prosthetic applications.

Specifically, over the past few years, many efforts have been made to develop devices in the form of gels/hydrogels and 3D structures with controlled morphology [27]-[29].

A first approach should involve nondegradable polymers and the design of customised prostheses using the reverse engineering approach.

The material–geometry design plays a crucial role in reproducing the mechanical behaviour of the native tissue, as well as the exact shape and size of the defect.

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Figure 1. The effect of the lay-down pattern. Stress–strain curves for additive manufactured PCL scaffolds tested up to a strain of 0.4 mm/mm.

Shell-core or multilayer devices can be designed, employing rubber-like and gel-like materials or the combination of conventional fabrication methods with additive manufacturing techniques.

In the current research, a second design strategy was reported, involving the development of customised porous structures and lattices with tailored morphological, biological, mechanical, and mass transport properties.

PCL, which is an aliphatic polyester, was considered as among synthetic polymers it has been widely studied for tissue engineering applications.

In the field of additive manufacturing, 3D fibre deposition, which represents a modified technique of 3D plotting to extrude highly viscous polymers, is a fused deposition technique in which the material is extruded/injected through a nozzle and appropriately deposited according to the selected lay-down patterns.

Scaffolds with different internal pore geometries (i.e. quadrangular, triangular, and complex polygonal) were designed adopting three lay-down patterns (0/90°, 0/60/120° and 0/45/90/135°, respectively), while the filament diameter, filament distance, and slice thickness were maintained as constant.

The stress–strain curves (Figure 1) obtained from the compression tests were similar to those reported for 3D fibre-deposited scaffolds [25], [26].

Table 1. Effect lay-down pattern on compressive modulus of 3D additively manufactured PCL scaffolds. Results are reported as mean value ± standard deviation.

|  |  |
| --- | --- |
| **Lay-down pattern** | ***E* (MPa)** |
| 0/90° | 39.1 ± 3.4 |
| 0/60/120° | 29.1 ± 2.8 |
| 0/45/90/135° | 20.1 ± 2.2 |

The compressive modulus is reported in Table 1 as the mean value ± standard deviation.

At fixed filament diameter, filament distance and slice thickness, the structures with a 0/90° pattern provided a compressive modulus that was significantly higher than those found for the other types of scaffolds (i.e. 0/60/120° and 0/45/90/135°).

Furthermore, with regard to the scaffolds with the 0/60/120° and 0/45/90/135° patterns, statistically significant differences were observed in terms of the compressive modulus.

It is well known that 3D fibre-deposited scaffolds are characterised by a column-like behaviour of the filament junctions under compression [25], [26].

Spanning from 0/90° to 0/45/90/135°, a larger contact area (i.e. fused area) was a clear consequence of a decrease in the amplitude of the deposition angle between the struts of adjacent layers. This led to a reduction of the local stress experienced by the structure, and in the scaffolds with a smaller amplitude of deposition angle, the filament sliding was easier, increasing the deformability of the structure.

*In vitro* biological analyses were carried out to investigate the influence of the adopted lay-down pattern on the behaviour of the AMSCs.

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Figure 2. Percentage of Alamar Blue Reduction for the PCL scaffolds at one, three, and seven days: the effect of the lay-down pattern. The data are reported as the mean value; the error bar represents the standard deviation.

Figure 2 reports typical results obtained from the Alamar Blue assay.

A redox reaction occurred in the cell mitochondria and the percentage of Alamar Blue reduction could be related to the number of viable cells. AMSCs may have survived and proliferated throughout the PCL structures as a significant increase of the Alamar Blue reduction was achieved over time.

Even though at day one no significant differences were found among the PCL structures, at three and seven days the scaﬀolds with a lay-down pattern of 0/90° showed values with a percentage of reduction of the Alamar Blue that were signiﬁcantly greater than those found for the other structures (*p* < 0.05).

Accordingly, a lay-down pattern of 0/90° significantly improved the cell viability/proliferation (Figure 2).

Thus, pore geometry influenced the cell viability and an increase in cell viability was generally evident when the number of deposition angles decreased.

To provide further insight into the effect of the lay-down pattern on cell morphology, CLSM was carried out.



Figure 3. Typical results from the CLSM analysis on the PCL scaffolds at one (A), three (B), and seven (C) days. Images of rhodamine phalloidin-stained cells/actin cytoskeleton (red). Scale bar of 100 µm.

As an example, Figure 3 reports typical results obtained at the end of one, three, and seven days of incubation.

Using the CLSM images, cell adhesion and spreading were further analysed to evaluate the shape factor.



Figure 4. Values of the shape factor obtained from CLSM images of the AMSCs on the PCL scaffolds with different lay-down patterns. Data are reported as mean value, and the error bar represents the standard deviation.

Values of the shape factor are reported (mean value ± standard deviation) at one, three, and seven days after the cell seeding (Figure 4).

A significant decrease of the shape factor was evident over time for all kinds of cell-scaffold constructs.

A decrease of the shape factor should suggest better cell adhesion and spreading since the lower the shape factor, the more elongated the cell [25].

However, in terms of the cell shape factor, no significant differences were observed among the scaffolds with different patterns.

Nevertheless, the obtained findings confirmed a strong influence of the lay-down pattern and, hence, of the pore geometry on the viability/proliferation of the AMSCs seeded and cultured on the PCL scaffolds.

In the literature, many studies frequently report the important roles of reverse engineering [30]-[33], computer-aided design (CAD), and finite element analysis [34]-[37], as well as the advances in methodologies of analysis and design strategies to develop smart devices for different applications [38]-[41].

For this reason, although the results suggest that a pattern of 0/90° would improve *in vitro* AMSC proliferation, the combination of lay-down patterns and, hence, of pores with different geometries within a single scaffold may represent a potential solution for the design of functionally graded structures that are capable of promoting optimal tissue regeneration while providing the required flexibility and strength.

1. conclusions

Taking into account the current scenario and a critical analysis on the breast reconstruction techniques, principles, and design methods for the development of 3D additively manufactured structures were proposed for breast reconstructive surgery.

In particular, an insight into the design and analysis of 3D scaffolds with different lay-down patterns was provided to improve surgery outcomes.

The possibility of fabricating 3D scaffolds with tailored architectures, functional and structural features by varying the lay-down pattern and, hence, the pore geometry, was stressed.

Biological analyses also demonstrated the effect of the lay-down pattern on the viability/proliferation of the AMSCs.

The reported experimental results, together with the image capture and analysis techniques (i.e. reverse engineering) would suggest the feasibility of developing customised structures and technical solutions in breast reconstructive surgery.

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