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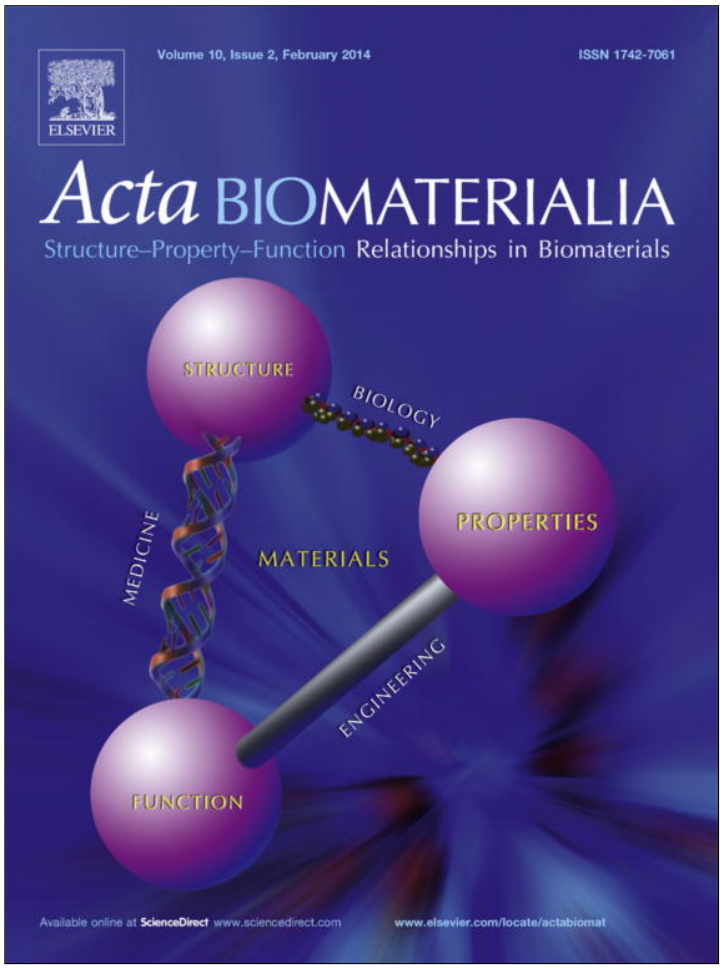

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Review

Current trends in the design of scaffolds for computer-aided tissue engineering

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ABSTRACT

Advances introduced by additive manufacturing have significantly improved the ability to tailor scaffold architecture, enhancing the control over microstructural features. This has led to a growing interest in the development of innovative scaffold designs, as testified by the increasing amount of research activities devoted to the understanding of the correlation between topological features of scaffolds and their resulting properties, in order to find architectures capable of optimal trade-off between often conflicting requirements (such as biological and mechanical ones). The main aim of this paper is to provide a review and propose a classification of existing methodologies for scaffold design and optimization in order to address key issues and help in deciphering the complex link between design criteria and resulting scaffold properties.

1. Introduction

In the last decade, tissue engineering (TE) has benefited from the development of additive manufacturing (AM) techniques, which have led to the production of free-form porous scaffolds with custom-tailored architectures. According to the latest ASTM standards, AM can be defined as "a process of joining materials to make objects from three-dimensional (3-D) model data, usually layer upon layer, as opposed to subtractive manufacturing methodologies" [1]. Unlike conventional subtractive processes that remove material from a 3-D block, additive manufacturing builds the final piece by the addition of material layers, starting from a 3-D computer model. The 3-D model is "sliced" into two-dimensional (2-D) layers, which are transferred to the AM apparatus for the fabrication of the final object. Commercially available AM techniques employed to fabricate scaffolds for tissue engineering applications include: selective laser sintering (SLS), stereolithography (SLA), fused deposition modeling (FDM), precision extrusion deposition (PED) and three-dimensional printing (3DP). Detailed descriptions of working principles, recent trends and current limitations of these techniques have been provided by several notable review articles [2–4].

One of the major obstacles to the diffusion of AM in TE has been long represented by the limited set of biomaterials that could be directly processed. However, recent advances in printing technologies, together with the synthesis of novel composite biomaterials, have enabled the fabrication of various scaffolds with defined shape and controlled in vitro behavior [5]. In this regard, the processing of hydrogels can be considered the main challenge, as these materials enable cell encapsulation during the deposition process, therefore representing an intriguing candidate for soft tissue engineering applications where the controlled spatial distribution of multiple biomaterials, cells and bioactive molecules [6] is needed for more precise mimicking of extra-cellular matrix (ECM) and a more natural healing process.

Together with the diffusion of additive manufacturing, a paradigm shift toward computer-aided tissue engineering (CATE) has occurred [7]. Exploiting advanced imaging tools, the design and fabrication of reproducible scaffolds, possessing desired porosity, pore shape and mechanical properties, have been greatly eased. The central role of scaffold microstructure in determining the functionality of the tissue-engineered construct, and hence of the newly grown tissue, has been pointed out. Consequently, an in-depth understanding of the effects of scaffold topological features is mandatory [8]. Precise engineering of microstructural elements has led to scaffolds with enhanced in vitro behavior with respect to those fabricated using traditional manufacturing methods, which permit the control of only overall scaffold morphological features, but not its microgeometry. Examples of such traditional manufacturing methods include porogen leaching, gas foaming and phase separation [9–11]. Since internal architecture greatly influences crucial factors for tissue regeneration, such as nutrient diffusion, cell adhesion and matrix deposition, scaffolds have to be carefully designed, keeping in mind case-specific mechanical, mass transport

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and biological requirements. However, customizing scaffold architecture to better suit conflicting requirements, such as biological and mechanical ones, remains a challenging issue. In this context, in silico validation using finite element analysis (FEA) has played a major role in the reduction of in vitro and in vivo experimental efforts. Furthermore, the reasonable accuracy of simulation results, in comparison with empirical tests, has led to the recent advancement of FEA to a predictive tool for a priori structural optimization. Indeed, scaffold design is becoming more and more an iterative process in which microarchitectures are created and refined in silico on the basis of tissue requirements and manufacturing process constraints. An overview of this design paradigm is reported in Fig. 1.

Given the volume of work in this field, many review papers focusing on different aspects of this topic have been published, including applications, advancements [5,12], and principal limitations [2] of rapid prototyping technologies in the manufacturing of scaffolds for tissue regeneration purposes. Other works specifically addressed emerging trends, such as the processing of hydrogels for soft tissues applications [6] or the engineering of functional organs by additive manufacturing [13]. Focusing more deeply on scaffold design, general overviews on advances related to the introduction of CATE [14,15] and successively to the implementation of computational modeling through the use of finite element methods, with particular regard to bone tissue engineering [16,17], have also been provided.

At present, however, none of the proposed AM approaches can be recognized as the gold standard for the design and optimization of scaffold architectures, not even for a specific target tissue. The main aim of this paper is to review the existing strategies for scaffold design and optimization in order to address key issues and the integration of recent achievements toward an efficient procedure for constructs engineering. A brief description of current trends in the design of additively manufactured TE constructs is also reported, together with an overview of the most challenging biological outcomes.

2. Classification of scaffold design architectures

2.1. Periodic porous structures

The physiological structure of native tissues is inherently heterogeneous and complex. Instead of trying to exactly reproduce their internal microarchitecture, literature is mainly focused on the creation of simplified models, functionally equivalent to the tissue to be repaired in terms of porosity and mechanical properties. In that vein, additively manufactured scaffolds have been designed using different elemental units with well-characterized mechanical and transport properties. The use of specific criteria for scaffold reconstruction has enabled a better understanding of the influence of each design parameter on the overall scaffold in vitro response. Indeed, starting from a small number of repetitive units, several different scaffolds with tunable architectures and properties have been designed.

Different design methods have been proposed, also in light of the chosen manufacturing process. For AM systems based on laser technology (e.g. SLA, SLS) and printing techniques (3DP, Theriform™), design approaches mimicking tissue architecture using 3-D unit cell tessellation have been applied, creating libraries of unit cells at different physical scales that can be assembled to form complex scaffold architectures. Boolean operations (e.g. intersection) performed on the acquired image of the defect site and the arranged stack of cellular units are required for the generation of scaffolds with patient-specific external shape along with controlled internal architecture [18]. Libraries of unit cells may be developed starting from solid, voxel and wireframe-based unit cells, created using either CAD software or image-based design approaches. Alternatively, a compact representation of scaffold basic elements has been recently pursued by the use of implicit surfaces [19].

However, the aforementioned methods cannot easily be applied to extrusion-based systems (e.g. FDM, PED, Bioplotter), which generate scaffold interior designs mainly consisting of regular, continuous patterns of rod-shaped elements.

In the following sections, a classification of the methodological approaches for the design of regular porous scaffolds is proposed. Architectures obtained by periodic replication of unit cells within a contour geometry will be referred to as "cellular structures", while architectures consisting of crossing rods, laths or other thin strips of material will be termed "lattice domains".

2.1.1. CAD-based methods

Most of the commercial CAD tools are currently based on solid or surface modeling systems, such as constructive solid geometry (CSG) and boundary representation (B-Rep). In CSG-based software, complex models are designed and represented combining standard solid primitives through Boolean operations. This process implies that it is possible to create only regular solids without dangling edges. However, design capabilities of CSG algorithms are often limited by the availability of solid primitives, mostly represented by simple geometric objects (cylinders, spheres or cubes). In the case of B-Rep, the solid is described through its boundaries, consisting of a collection of vertices, edges and loops, with no explicit relation existing among them. For this reason, a preliminary check is required to verify that either gaps or overlaps do not exist among the boundaries [20]. B-Rep models require more storage space than CSG ones, but less computation time. For this reason, as objects become large or with fine internal architectures, their size dramatically increases, and they become very hard or impossible to visualize and manipulate.

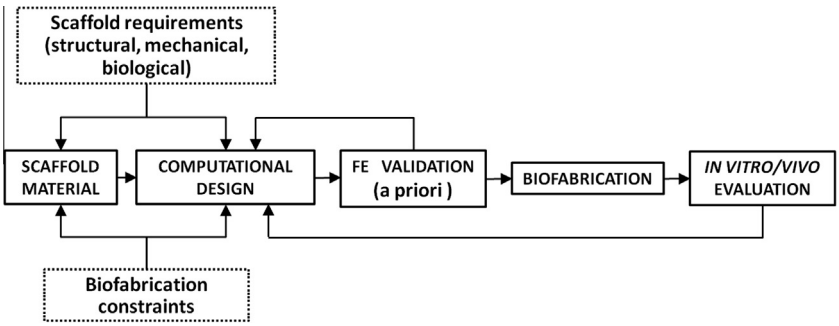


Fig. 1. Flowchart of the key steps in the design of scaffolds for CATE, showing the introduction of FEA as a predictive tool for a priori optimization.

Commercial software based on the above-cited modeling principles – such as NX (Siemens PLM Software), CATIA (Dassault Systèmes), Pro/Engineer (PTC), SolidWorks (Dassault Systèmes) and MIMICS (Materialize Gmbh) – have been frequently employed for the design of 3-D structures [11,21]. Therefore, many CAD-based unit cells, derived from different primitive geometries, have been developed, completely characterized and combined into scaffold microarchitectures. Among the others, several polyhedral shapes, including Platonic and Archimedean solids, have been investigated and selected as basic building blocks, either creating void spaces in the continuous domain by the use of Boolean intersections with primitive volumes or constructing wireframe approximations [22,23].

In order to reduce the time-consuming and tedious manual cell design and assembling, Chua et al. [24] and Cheah et al. [25] have developed a parametric library of scaffold structures [24,25] and an algorithm to automate the entire process of matching desired anatomical shapes [26]. This collection of unit cells, also known as CASTS (computer-aided system for tissue scaffolds) [27], has been refined with the addition of a tool that automatically calculates basic information such as pore size, porosity level and surface-area-to-volume ratio starting from input design parameters. Furthermore, a database that correlates porosity levels to compressive stiffness values, using poly(ε-caprolactone) (PCL) as a reference material, has been recently compiled [28], with the aim to extend CASTS to the design of more complex functionally graded scaffolds. Thus, by changing few structural parameters, different combinations of pore sizes, porosity values and mechanical properties can be obtained and easily modified upon application requirements.

However, to overcome the limitations of most CAD-based libraries (i.e. simple pore-making elements), slightly different unit cells composed of more intricate bio-inspired features have been introduced [29,30]. For instance, starting from computed tomography or magnetic resonance imaging (CT/MRI), trabecular architectures have been derived from different portions of human bone and converted into CAD models. Feature primitives, e.g. plate-like (for femur), rod-like (for spine) and hybrid primitive (for iliac crest) have been obtained and combined into a library (Fig. 2), which has been successfully applied to the representation of heterogeneous biological tissues [31]. Other solutions addressing the construction of more complex internal scaffold microstructures will be described in the following.

Although CAD-based methods provide a powerful tool for the modeling of 3-D scaffold geometries, and represent the most widely employed design approach, they are hampered by their poor performance in reproducing items such as biomorphic (i.e. resembling naturally occurring shapes) porous structures and non-Euclidean solids [20]. In addition, despite the efforts to considerably automate modeling procedures by developing dedicated subroutines [26,27], the control over the resulting biomechanical properties of the scaffolds is still poor.

2.1.2. Image-based design

Image-based design represents an alternative method, initially proposed by Hollister et al., for the design and manufacture of patient- and site-specific scaffolds with controlled internal architectures [32]. This approach combines currently available imaging, image processing and design software with solid free-form fabrication techniques to simplify and accelerate scaffold design procedure. Image-based methods produce scaffold microarchitectures by the intersection between two 3-D binary images (i.e. in which the voxel values are Boolean, and correspond to "solid" or "void"), one representing the shape of the defect to be reproduced (obtained from thresholding of medical imaging data), and the other consisting in the stacking of a binary unit cell (implementing the pore motif to be patterned in three dimensions).

Starting from this tool, both empirically derived and bio-inspired geometries have been generated. Empirically derived geometries include designs in which geometric shapes (cylinders, spheres and similar entities) are created in the unit cell to infer a regular channel of pores within a scaffold. Similarly, unit cells exhibiting randomly arranged pores can be designed by the use of a random number generator to set voxel state. Bio-inspired unit cells can instead be obtained by setting voxel values within the unit cell domain according to patterns derived from medical imaging. The feasibility of the proposed approach has been demonstrated by manufacturing scaffolds for craniofacial reconstruction, such as a mandibular condyle, an orbital floor and a generic mandibular defect, which have been used for in vivo tests in animal models [33,34]. The integration of the image-based approach with topological optimization algorithms has been pivotal to obtain scaffolds that match conflicting requirements on functional and mass-transport properties [35,36].

If compared to CAD-based methods, image-based ones guarantee much faster creation of scaffold architectures, due to their direct compatibility with CT and MRI imaging. As previously underlined, traditional CAD-based techniques, which represent solids via geometric functions, require segmentation algorithms to convert 3-D voxel-based images into their CAD representation. Furthermore, image-based techniques allow the creation of multiple datasets at different resolutions for the same scaffold, which makes it possible to use different length scales for the design of the external anatomical shape (macro) and its interior porous architecture (micro). However, the major limitation of image-based methods is represented by dimensions of the datasets and constraints related to the physical resolution at which materials can be processed.

2.1.3. Implicit surfaces

Implicit surface modeling (ISM) is a highly flexible approach, recently proposed as a valid tool for the generation of cellular structures providing a compact representation of potentially complex surfaces [19,37]. ISM allows scaffold architectures to be easily described using a single mathematical equation, with freedom to introduce different pore shapes and architectural features, including pore size gradients. Recently, attractive candidates for the design of biomorphic scaffold architectures have been modeled through implicit functions belonging to the large class of periodic minimal surfaces. The occurrence of minimal surface geometries in nature – such as in beetle shells, weevils, butterfly wing scales and crustacean skeletons – hinted at their usefulness in scaffold design [38]. Thus, to overcome the limitations of existing scaffold fabrication strategies that produce sub-optimal porous labyrinths with contra naturam straight edges, continuous and optimized unit cells, based on minimal surfaces, have been introduced.

A premier attempt in the computer-controlled fabrication and mechanical characterization of tissue engineering scaffolds based on triply periodic minimal surfaces (TPMS) has been presented by Rajagopalan and Robb [39]. Unit cells based on Schwartz's Primitive (type P) TPMS were assembled in a coterminous seeding-feeding network and additively manufactured. High-precision fabrication of TMPS-based scaffolds – such as Schwarz's Diamond (D) and Schoen's Gyroid (G) – has been elsewhere demonstrated [40–42]. More complex structures with different volume fractions of pores as well as functional gradient architectures have been successfully designed and manufactured by SLS [19] and SLA [43]. Examples of implicit-surface-based architectures are illustrated in Table 1.

A positive effect of these morphologies on cell migration and tissue ingrowth has been testified both experimentally and computationally [10,44]. In a recent paper by Melchels et al., the influence of pore shape on cell seeding efficacy and static culture

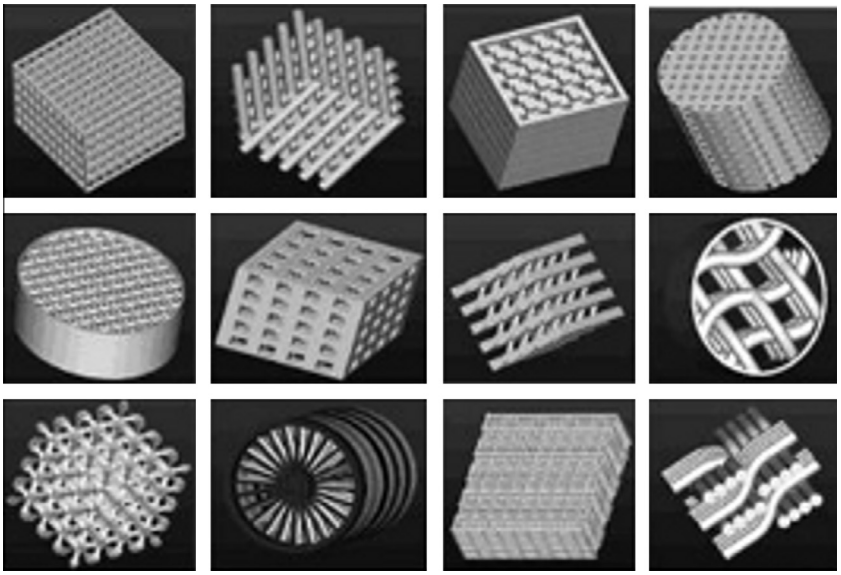


Fig. 2. A library of unit cells based on different CAD-based primitives (reproduced with permission from Ref. [31]).

outcome has been experimentally assessed by comparing a gyroid architecture (fabricated by SLA) [43] with a random-pore architecture resulting from salt leaching [10]. It was proved that gyroid scaffolds could be more easily wetted and intruded by a cell suspension, resulting in a more homogeneous cell distribution and a deeper cell colonization. Although the two scaffolds showed comparable porosity and pore-size distribution values, the gyroid type revealed more than 10-fold higher permeability, due to the absence of size-limiting pore interconnections. These results suggest that the use of AM for scaffold fabrication can lead to a more homogeneous cell distribution after seeding and facilitate static culturing, with sustained nutrient and oxygen supply.

However, specimens designed and manufactured in all the above-mentioned works are characterized by simple cubic or cylindrical outer shape, while a patient-specific one is needed for clinically relevant applications. To this end, a design methodology for constructing a pore network composed of TPMS-based unit cells within an arbitrary complex anatomical model has been recently developed and successively optimized by Yoo et al. [45,46]. In their first work, 3-D models representing the outer shape of different human bones (femur, tibia and iliac crest) were divided into multiple hexahedral elements; these were used to create a pore network by a TPMS algorithm. A variety of complex scaffolds has been fully automatically generated with the required microarchitecture, while keeping constant the external geometry of the anatomical model. However, if high quality rendering is required, the number of voxels needed to represent large surfaces with small details can easily exceed memory capacities of personal computers. To overcome such limitation, the proposed method has been further refined in terms of computational efficiency and successfully tested on several patient-specific geometries derived from real CT data.

Hence, the TPMS unit cell library, combining the strengths of both traditional CSG and image-based design, provides a computationally efficient method for the modeling and fabrication of computer-aided manufactured scaffolds.

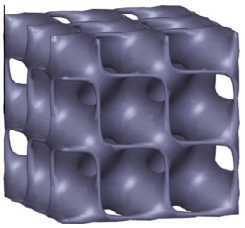
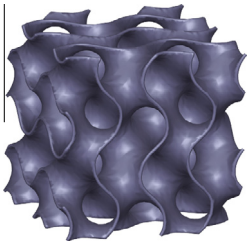
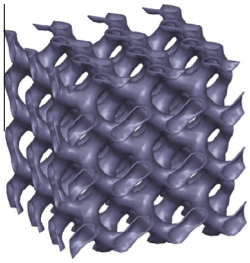
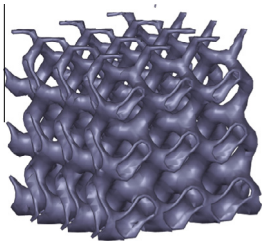
2.1.4. Space-filling curves

The previously reported design methods can hardly be coupled to extrusion-based manufacturing techniques. Such techniques consist in the microextrusion of a small-diameter polymeric filament by means of a heated extruder, terminating with a nozzle having an orifice diameter in the hundreds of microns range. The fabrication process involves the subsequent deposition of several biopolymeric layers, which adhere to each other by virtue of the processing temperature, while retaining their shape. According to the type of feedstock (filament, powder and granulate) and the extrusion mechanism (mechanical or pneumatic), extrusion-based techniques have been classified as fused deposition modeling, precision extrusion deposition, bioplotter and other variants [47].

Considering the inherent manufacturing constraints, patterns that do not cause filament intersection (to be laid in a layer-by-layer fashion) and that require the minimum number of start and stop points (to avoid the agglomeration of material due to the delayed response time) have been looked for. The presence of a repetitive pattern in the scaffold design has been also considered as a positive factor to simplify the deposition process. As a consequence, the simplest scaffold, which can be manufactured using extrusion-based processes, has cube-shaped pores formed by orthogonal rasters. More complex patterns can be obtained by changing the deposition angle between adjacent layers (Fig. 3A). Because of the regular repetition of identical pores they exhibit, these geometries have been named honeycomb-like patterns [48]. By varying architectural parameters, such as fiber diameter, spacing, layer thickness and stacking direction, 3-D scaffolds with controlled porosity and tailored mechanical properties can be easily obtained. Indeed, structural modifications affecting scaffold porosity have an influence on the mechanical properties, in agreement with an experimentally validated power-law relationship between porosity and compressive stiffness [48]. However, for constant porosity, mechanical properties can be also tuned by modifying the deposition pattern, as the number of contact points between the struts defining a pore varies with the deposition angle [49,50].

Several studies have investigated the effects of printing parameters and deposition patterns on the final scaffold mechanical behavior under different experimental conditions (static or dynamic loading [51,52] performed in physiological and ambient environment [53,54]). For example, Woodfield et al. demonstrated that, for polymeric scaffolds obtained by 3-D fiber deposition (3DF), a homogeneous fiber spacing resulted in higher equilibrium modulus (i.e. compressive modulus at equilibrium after imposed strain) and dynamic stiffness, if compared to a staggered fiber spacing, even though the two samples had almost identical porosity [51]. Similarly, other works point out that if the orientation angle between two consecutive layers varies (or the number of layers

Table 1
Examples of implicit-surface-based architectures.

Architecture	Trigonometric function	3-D model
Diamond	$D : \sin(x)\cdot\sin(y)\cdot\sin(z)+\sin(x)\cdot\cos(y)\cdot\cos(z)+\cos(x)\cdot\sin(y)\cdot\cos(z)+\cos(x)\cdot\cos(y)\cdot\sin(z)=0$	
Gyroid	$G : \cos(x)\cdot\sin(y)+\cos(y)\cdot\sin(z)+\cos(z)\cdot\sin(x)=0$	
Gyroid, high porosity	$G_{HP} : \cos(x)\cdot\sin(y)+\cos(y)\cdot\sin(z)+\cos(z)\cdot\sin(x)-k=0$	
Gyroid, gradient architecture	$G_{GA} : \cos(x)\cdot\sin(y)+\cos(y)\cdot\sin(z)+\cos(z)\cdot\sin(x)-k'z-k''=0$	

printed with the same orientation on top of each other is doubled), a different mechanical behavior is obtained, even with a constant porosity [52,55]. More specifically, a change from single to double layer configuration and from 90° to 45° rotation between consecutive layers was reported to infer lower mechanical properties [52]. Furthermore, a significantly lower stiffness under compressive loading was measured in scaffolds with more complex honeycomb pores (i.e. triangular vs. polygonal ones) [53]. Nevertheless, no significant difference in the in vitro cell response was observed for different pore architectures and stacking directions [53,55].

An alternative route to tailor scaffold mechanical properties consists in the fabrication of hybrid structures, obtained by the incorporation of reinforcement materials (such as particulate) into the fibers or by the deposition of different materials in the layer-by-layer strand configuration. This approach can ensure the achievement of the desired mechanical behavior together with other conflicting requirements in a single multifunctional structure [56,57].

The aforementioned microstructural layouts have been used for the processing of scaffolds in various biocompatible polymers and composites, resulting in successful candidates for different applications, such as bone [58–60], osteochondral substitutes [61,62] and lumbar interbody fusion implants [63]. Despite the positive results obtained, low cell seeding efficacy and heterogeneous cell distribution are often considered among the major drawbacks of extrusion-based techniques. Some authors have hypothesized that the presence of tortuous conduits inside the scaffold, increasing the residence time of cells within the scaffold and the likelihood of contact between them, should enhance seeding efficacy. As an example, Lee et al. [64] demonstrated that a staggered structure, introducing a more tortuous conduit inside the scaffold, increased cell proliferation (up to 30% after 1 week of culture) with respect to a lattice scaffold with the same pore size and porosity values. Furthermore, in a recent study, Sobral et al. evidenced that, under static culture conditions, seeding efficacy, as well as cell distribution, was significantly increased in structures with a pore size gradient along one direction. The graded porosity was obtained by printing layers in a 0/90° fashion, and by varying in-plane fiber spacing along the Z direction [65]. Table 2 summarizes the most notable solutions that were adopted to enhance mechanical behavior and in vitro response of scaffolds produced by extrusion-based techniques.

An improvement to simple rod patterns for the design of scaffold internal architectures has been obtained by the use of space-filling curves (Fig. 3B). They are defined as curves that fill up the given space in a particular order, by either recursive changes of trajectory or passing through every given point in the defined space [66]. Due to the restrictive features of extrusion-based techniques, continuous, self-similar, non-intersecting curves that satisfy all the above-mentioned constraints are particularly attractive. This special class of space-filling curves, named fractal space-filling curves,

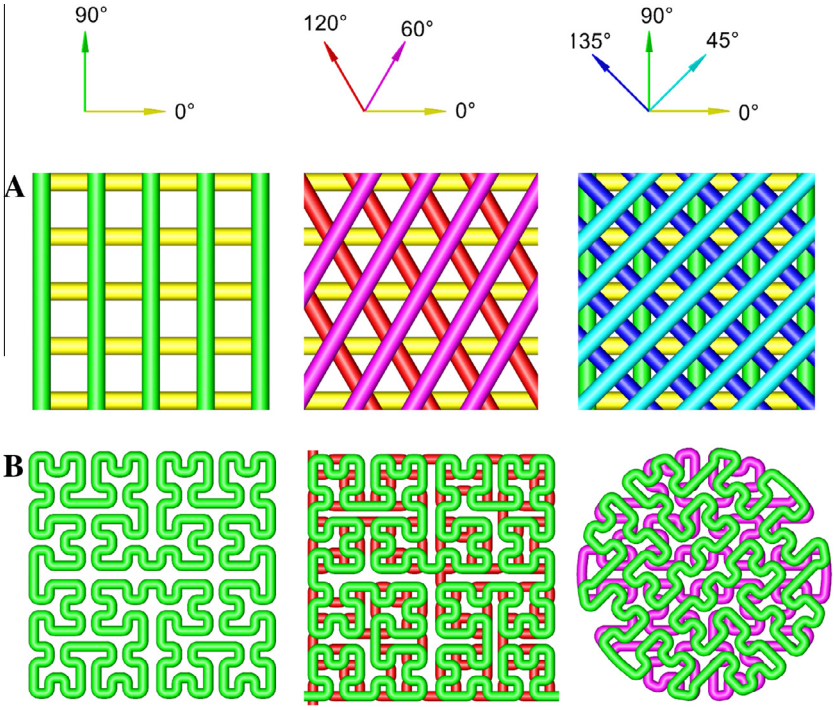


Fig. 3. Lay-down patterns with honeycomb pores (A) and Hilbert recursive curves (B).

can be mathematically generated by starting with a very simple pattern that grows through the recursive application of a small set of rules, described by a generative grammar.

A proof of principle of how space-filling fractal curves can be applied to TE scaffold design has been proposed by Starly and Sun [67]. With the aim to provide a better substrate for cell organization, two fractal space-filling curves (Hilbert and Sierpinsky) were separately used for the fabrication of a circular scaffold by PED. This concept was further extended to the design of a scaffold for bone TE with subject-specific external shapes along with location-controlled porosity. Kumar et al. investigated six different space-filling fractal curves in order to obtain gradients of material within selected scaffold regions. By changing the curve type and its iteration level, the desired porosity was obtained [68].

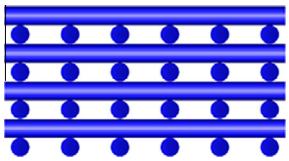
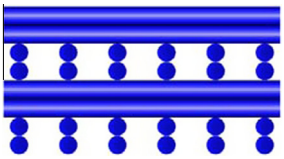
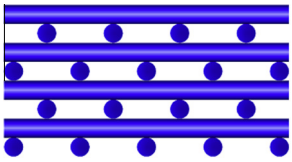
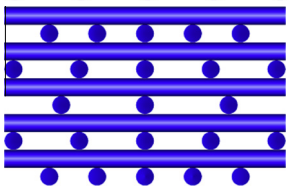
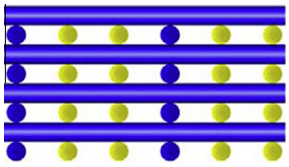
2.2. Irregular porous structures

The majority of constructive approaches are targeted to design the internal geometry of a scaffold by filling it with the periodic repetition of regular unit cells. The advantages of such periodic porous structures (PPSs) consist in their easier modeling and fabrication, as well as in the possibility of predicting their structural properties. Even though the geometry of unit cells can be relatively sophisticated, PPSs are still regularly and repeatedly packed in the geometric domain. Due to such a regularity and periodicity, it is very difficult to exert local control on pore shape, size and distribution, since a minute modification of the unit cell will turn into global changes to the entire structure. Furthermore, currently available CAD tools are often not suitable to reproduce the complex arrangement of natural structures [69].

As a consequence, biomimetic design has been introduced and extensively used as an alternative method for the modeling of irregular porous structure. The complex microstructures of several natural materials, such as wood and bone, are known to be optimized for withstanding functional loads. Hence, the design of synthetic scaffolds inspired by those high-performance structures has gained increasing interest. For example, the microstructure of cuttlebone, which matches high compressive strength with high porosity, has inspired the design of cellular optimized architectures [70]. Biomimetic 3-D models, which accurately mimic the interior configuration of natural bone, have been also fabricated, starting from computer tomography and histological data [71]. However, such a faithful reproduction is, in most cases, not strictly necessary: the functional properties of natural architectures are indeed to be caught in a concise and efficient manner. Thus, a simpler approach for the achievement of a biomimetic design is to mimic the functionality of tissues or organs, varying the porosity of different regions according to a natural reference model. Simple observations of tissues, such as bone and skin, show that these structures exhibit well-organized architectures, not only anatomically, but also in terms of biological and mechanical characteristics. Scaffolds trying to reproduce such architectural cues by the introduction of material gradients are termed functionally graded scaffolds (FGSs) [72].

The design of FGSs requires the introduction of new computer-aided tools to obtain the desired porosity distribution profile in a continuous and interconnected way throughout the entire geometry. Discontinuities at the interface of two adjacent regions and disconnectivities in terms of deposition path planning, often detected in scaffolds with variational porous architectures [73], negatively affect fluid flow, nutrients and waste transport, also resulting in local stress concentrations. As such, there is a pressing need for strategies to optimize the design of scaffolds with appropriate continuous functional gradients. To this aim, an optimization method has been recently implemented by Khoda et al. to connect differently spaced tool paths, improving continuity and connectivity between different functionally graded regions. The methodology has been tested on three different models of both soft (aorta) and hard (vertebrae, femur) tissues, starting from a biomimetic pathway of porosity regions derived from MRI medical data [74]. An advance over this approach has been proposed by the same group with the aim to extend its use to the generation of a continuous tool path for tissue scaffolds containing hollow features [75,76] and for applications requiring a spatially graded distribu-

Table 2
Different fiber arrangements implemented using extrusion-based manufacturing techniques.

Typology	Arrangement	Schematic diagram	Refs.
Single material scaffolds	Homogeneous fiber spacing		[48,51,52,65]
	Double layer configuration		[52]
	Staggered fiber spacing		[51,55,64]
	Pore size gradients		[65]
Hybrid scaffolds	Bi/multimaterial		[56,117]

tion of biomaterials and biomolecules [77]. Alternatively, a set of fractal space-filling curves has been used by Pandithevan and Saravana Kumar to obtain a scaffold for bone TE with patient- and site-specific controlled porosity. In this work, a method for the reconstruction of scaffolds with porosity patterns derived from CT data was developed, with the aim to mimic the stiffness of native bone [78].

Efforts in the direction of FGS have been made also starting from CAD-based unit cells. Notable examples are provided by libraries in which the connection between unit cells has been handled thanks to the introduction of common interfaces [79]. For polyhedral units, a common connector feature (a torus shape) was added at every cellular side/facet in order to allow a smooth change between different microarchitectures and to ease mating between adjacent unit cells. As a case study, the methodology was tested on the design and additive manufacturing of a scaffold for vertebral body replacement [80]. Furthermore, to simplify the design of CAD-based FGS, a complete database that correlates properties (namely structure and porosity) of several unit cells to their corresponding compressive stiffness values has been developed [28]. Nevertheless, if the reproduction of biological tissue interfaces is concerned (e.g. bone/cartilage interface in osteochondral TE), existing systems have not been able to generate suitable designs, as the relationship between tissue requirements and scaffold structural parameters is still not well established. In this case, heterogeneous materials and architectures may be needed to provide functional alterations throughout the scaffold domain. Indeed, although the engineering of bicompartmental architectures [81–83] and the manufacturing of functionally graded structures [84] have been separately experimented, a close integration between the two has not yet been achieved.

Besides the aforementioned approaches, stochastic and Voronoi models have been used to mimic the randomness of natural tissues and to translate it into scaffold design. Scaffolds with heterogeneous simple-shaped pores, biomimetically distributed according to a given porosity level, are among the most notable results of the implementation of stochastic methods [85,86]. Starting from randomly packed microspheres, Lal and Sun developed a computer modeling approach for the design of 3-D bone grafts with controlled porosity. To determine the number of microspheres packed in a synthesized scaffold, three different packing models were used: two extreme microsphere-packing models (minimum- and maximum-density packing) and a statistical one [87]. However, although the method achieved an irregular pore distribution, the basic pore-making element was generally represented by a simple sphere. To overcome this limitation, a hybrid Voronoi-spline representation has been recently combined with a random colloid-aggregation model [88], with the aim to define pore morphology and location, mimicking the natural process involved in the generation of porous structures. More specifically, Voronoi tessellation was implemented to partition the space into a collection of regions, which were then opportunely selected and merged together according to the random colloid aggregation model, while B-spline curves were employed to represent the boundaries of the irregular-shaped pores [69]. The proposed method has been further extended to implement graded pore sizes and pore distributions [89].

Finally, other approaches for the creation of heterogeneous porosity within a solid model make use of volumetric mesh generators, derived from finite element software tools [90,91]. In particular, a novel route to produce patient-specific breast prostheses in a highly automated manner has been developed. In this case, a tetrahedral mesh was created within a personalized breast 3-D model

and used to design a completely interconnected strut-based microarchitecture by arranging material along the sides of mesh elements [91].

3. Design optimization strategies

For years, a "trial-and-error" approach has been adopted to validate scaffold microarchitectures, with ex post modifications being made to an existing design on the basis of in vitro or in vivo results. In silico experiments offer the possibility to widen the range and number of design parameters within a simulation as needed to identify the most suitable configuration for the replacement of a desired tissue [16].

Several approaches reported in the literature are devoted to the modeling of scaffolds with subject-specific external shapes and intricate internal architectures. Among these, analytic methods provide an estimate of the global mechanical behavior by means of empirical relationships between structural parameters and mechanical properties. More recent studies use FEA for post hoc investigation of scaffold properties in order to modify architectural parameters according to specific requirements. AM processes, requiring the generation of precise digital representations of the scaffolds to be manufactured, endow a naïve interface to FEA environment. In particular, CAD design can provide an accurate input model of scaffold morphology at the microstructural level. However, the influence of discrepancies between the final shape of additively manufactured objects and their intended design – due to issues arising from the fabrication process (surface roughness, micropores, etc.) – still remains poorly understood [92,93]. As a consequence, some authors have opted for applying FEA to the μCT reconstructions of additively manufactured scaffolds, rather than relying on their CAD model [94,95]. Despite the standing debate on the accuracy of CAD-based modeling, reasonable agreement with experimental results has been demonstrated for several porous additively manufactured scaffolds [43,96]. FEA has been successfully used for the prediction of mechanical properties of AM polymeric and composite scaffolds. Good agreement with experimental tests has been found in the case of PCL/hydroxyapatite scaffolds manufactured by SLS [97].

Several computational studies have been focused on determining the effect of pore topology on the resulting mechanical properties, while only a few have tried to provide a deeper insight into how pore morphology affects failure mechanisms [98]. Additionally, pore morphology has a profound influence on mass transport properties, which in turn affect cell viability, proliferation and, ultimately, overall tissue regeneration. Hence, an accurate and efficient prediction of scaffold mass transport properties would be important for an efficient scaffold engineering process [99]. As with mechanical properties, physiological values can be regarded as a starting point for defining design targets [100]. Also in this case, computational methods have integrated empirical evaluation with the aim to provide a more rigorous approach to determine mass transport characteristics of AM scaffolds.

As long as diffusion is concerned, AM scaffolds can be described in terms of effective diffusivity, which is a function of porosity-related adimensional parameters (pore volume fraction, pore constrictivity and tortuosity). The application of FEA to the calculation of effective diffusivity for AM scaffolds offers a more rigorous approach to determine the diffusion of oxygen and nutrients as a function of scaffold microstructure, as demonstrated by Jung et al., who calculated effective diffusion coefficients for several AM scaffold geometries with different internal porous patterns [101].

However, although an open permeable pore network sensibly facilitates scaffold wetting and improves oxygen and nutrient transport, it might not be enough to ensure adequate, long-term cell viability. It is commonly recognized that static seeding and culturing, although still widely used in laboratory settings, are characterized by low seeding efficacy and inhomogeneous cell distribution. Moreover, in 3-D static culture settings, the majority of oxygen and nutrients are consumed by cells adhering to the surface of the scaffold, while cells in the interior are challenged by oxygen deprivation and hypoxia-induced death [102]. Thus, perfusion of medium has been suggested as a solution to increase the survival rate of cells within porous scaffolds, resulting in more homogeneous tissue engineered constructs. This accounts for the widespread diffusion of perfusion bioreactors, where a fluid is forced to continuously flow through the pores of the scaffold, enhancing mass transfer [16]. However, an accurate knowledge of the fluid flow inside and around the scaffold is mandatory to precisely control culture conditions and cell behavior. Increased flow rates can create large shear stresses, which can adversely influence cellular metabolism and even damage or detach seeded cells. At the same time, the application of proper fluid shear stresses has been proved to promote cell proliferation, differentiation and matrix deposition within 2-D and 3-D environments, allowing a more realistic in vitro study of cell–biomaterial interactions [103]. For this reason, computational fluid dynamics (CFD) has been applied to AM scaffolds geometries (either starting from CAD designs or their μCT reconstructions) with the aim of investigating the permeability of regular AM scaffolds for skeletal tissue engineering [104]. The introduction of these advanced characterization tools has fuelled interest toward the quest for new scaffold microstructures optimized in terms of mass transfer of nutrient and gases. Several works have used CFD models to study the local shear stress distribution within AM scaffolds [44,105,106], while only a few have tried to perform a correlation between CFD results and scaffold microarchitectural parameters [107,108]. In particular, Yan et al. pointed out the effect of controllable parameters in scaffold fabrication (i.e., the diameter of scaffold strand and the distance between two strands) and the cell culture process (i.e., the flow rate) on the distribution of shear stress within lattice-type AM scaffolds under perfusion and non-perfusion conditions [107]. Similarly, Yao et al. identified the relationship between internal shear stress distribution and pore morphology for different unit cells using the ellipsoid geometry as a basic pore-making element and its major axis as the shape control parameter [108]. An advancement in this field has been achieved by Melchels et al., who took advantage from gradients in shear rate, induced by anisotropic graded architecture, to modulate the distribution of seeded cells, with the ultimate goal of engineering complex 3-D tissue constructs with biomimetic zonal organizations [109].

Going beyond the computation of scaffold properties as derived from a defined microstructure, in silico modeling has been recently combined with topological optimization for the a priori design of new optimized scaffold architectures [92]. As previously evidenced, conflicting requirements have to be simultaneously addressed in the design of porous scaffolds, since the large porosity required for increased mass transport negatively affects mechanical properties. For this reason, the development of design strategies capable of optimal trade-off between these two opposite needs is mandatory to satisfy both mechanical and biological requirements. Topology optimization approaches involve the determination of features such as location, number and shape of "holes", as well as connectivity of the domain, in order to find the best use of material within a structure subjected to either single or multiple load distribution [110]. Thus, instead of relying on designers' ability and a predefined library of unit cells, topology optimization was utilized to design scaffold unit cells/architecture that attain desired properties under given constraints. These approaches have usually been applied to the optimization of mechanical properties with a constraint on porosity [111] as well as to the

maximization of scaffold permeability [36,112]. A milestone work in this field was performed by Hollister [36], who designed microstructures with optimized permeability for cell migration and mass transport and with mechanical properties matching those of natural bone tissue. Almeida and Bártolo [113] have recently developed an innovative tool combining CAD-based modeling and topological optimization, providing an a priori control of the mechanical properties as a function of porosity and scaffold architecture. Instead of starting from a predefined unit cell, the tool starts from a dense non-porous block of material, searching for a topologically optimized scaffold unit according to porosity and stiffness requirements. As a further progress in scaffold optimization, a load-adaptive scaffold architecturing (LASA) algorithm has been proposed, with the aim of designing scaffold microarchitectures which are topologically optimized in terms of mechanical properties for specific loads and boundary conditions. Instead of considering the design domain filled with a cellular structure and trying to optimize the repeating unit cell, the LASA method starts from a dense continuous solid model and generates a trabecular architecture, by engineering the material arrangement within the considered volume [114]. In Fig. 4, a schematic diagram of LASA algorithm is reported.

The above cited works testify the intensive research that is being carried out in pursuing the optimization of scaffold design. Nevertheless, for biodegradable scaffolds, an initial optimal design may not guarantee the desired behavior due to in vivo degradation and neo-tissue ingrowth. Although the concept that scaffolding material is expected to gradually disappear over time has been widely accepted, how matrix degradation affects tissue ingrowth and biomechanical capability of the tissue–scaffold system for better promoting the regeneration outcome is still under study. In this framework, FEA has been combined with empirical investigations to evaluate the effects induced by the degradation process, especially in terms of mechanical functionality. In addition to in vitro studies regarding scaffold degradation [115], the significant influence of several architectural parameters on the in vivo degradation profile has been recently attested within a given material [116]. Furthermore, scaffolds with an optimized set of mechanical properties together with an appropriate degradation kinetics have been successfully produced using an innovative numerical optimization algorithm [117] that combines different materials to engineer a scaffold matching the imposed requirements.

However, interest is moving toward the integration of matrix degradation with tissue regeneration in order to better understand how they can affect in vivo scaffold properties over the healing time. In this context, mechanobiology – the discipline studying the mechanisms according to which cells sense and respond to mechanical forces [17] – has attracted great interest. Adachi proposed a framework for optimal design of homogeneous porous scaffolds, taking into account bone tissue regeneration and scaffold degradation for a 3-D scaffold unit cell [118]. In a similar manner, Erkizia et al. proposed an algorithm for the simulation of the AM scaffold degradation process using a Monte Carlo algorithm, which takes into account the curvature at the scaffold/liquid interface [119]. A different approach based on the mechano-regulation algorithm has been used by Byrne et al. [120] in order to study the influence of several factors (e.g. porosity, Young's modulus and dissolution rate) on tissue differentiation and bone regeneration for a regular scaffold subject to uniaxial loading conditions. Furthermore, since the effect of vascular supply also plays a critical role in bone formation, a computational model based on mechanical environment and local vascularity has been also developed [121]. As a result, inclusion of angiogenesis in the simulation led to a more heterogeneous cell distribution, in close agreement with histological observations.

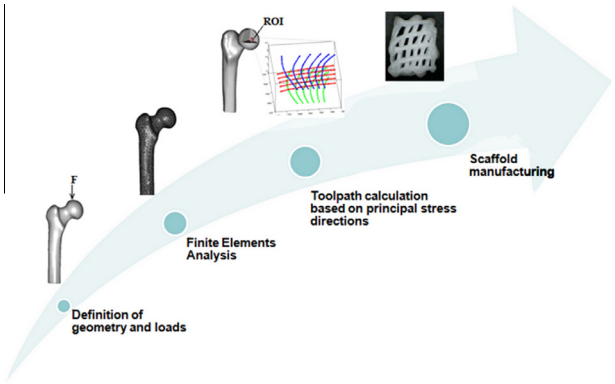


Fig. 4. LASA algorithm as an example of a priori topological optimization.

However, how the scaffold architecture and degradation could continuously alter the mechanical response of a construct and consequently determine the tissue regeneration outcome is still unclear. Only recently, Chen et al. have explored the performance of several topologically optimized scaffolds with different combinations of stiffness and permeability, assessing their capabilities of stimulating neo-tissue growth in a biodegradation framework [122]. In this work, the evolution of scaffold degradation (in terms of stochastic hydrolysis) was merged with tissue ingrowth (modeled through the mechano-regulatory theory) in a time-dependent manner, demonstrating the impact of architectural features on tissue regeneration outcome.

4. Challenges in computer-aided tissue engineering

Each tissue exhibits peculiar features in terms of function, cell phenotype, ECM components, etc., which have to be taken into account in the scaffold design phase, in order to recapitulate the tissue-specific microenvironment. For example, while high diffusivity and permeability values are mandatory in several tissue engineering applications, limited mass transport is required for cartilage regeneration due to the avascular and low metabolic rate of this tissue [123]. As mentioned in the previous section, design is further complicated by the fact that requirements for mechanical stiffness typically conflict with those for mass transport. Moreover, although the importance of design parameters including scaffold porosity, pore shape, dimension and interconnectivity is generally recognized, no consensus on the ranges of optimal values has yet been achieved [124].

What emerges clearly, however, is that the manner in which a material is arranged and structured at the micro- and nanoscale modulates cell signaling and organization, playing a pivotal role in cell–scaffold interaction [125]. Thus, a continuous challenge in scaffold engineering is to optimize microarchitectures to precisely regulate cell behavior. A widely adopted strategy consists in tailoring scaffold architectural features according to the needs of the selected cell source. Cell type and dimension, together with cell activity and phenotypic expression, have to be taken into account, since different responses to the same scaffold architectural features have been registered when starting from different cell sources [126–128]. In this regard, the strong trend regarding the use of mesenchymal stromal cells (MSCs) for the fabrication of cellularized constructs has to be considered. Because of the multilineage differentiation capability of MSCs, it is crucial to properly select architectural parameters in order to define the context for self-renewal and differentiation in a fashion similar to their native niches. Physical factors, including porosity, pore size, scaffold interconnectivity and mechanical strength, have been shown to influence the osteogenic differentiation of MSCs seeded on scaf-

folds both in vitro and in vivo [129]. It has also been demonstrated that such properties might impact the architecture and the amount of in vivo bone formation [129,130]. Similarly, a strong effect of mechano-structural features, such as scaffold geometry and stiffness, has been observed for the cardiomyocyte commitment of cardiac progenitor cells (CPCs) cultured upon AM scaffolds [128].

The aforementioned works testify the ongoing efforts to gain a deep understanding of how to guide and stimulate the appropriate cellular response. Fig. 5 reports the main biological aspects that have to be considered during scaffold engineering in order to implement a "biologically informed" design [131,132].

To date, additive manufacturing of clinically sized tissues with high anatomical precision has been primarily demonstrated for hard tissues such as bone and cartilage [58,133,134], while soft tissue applications, albeit strongly emphasized for their pivotal role in the possible regeneration of functional organs, still represent a minority in the AM research scenario.

4.1. Bone

Although a broad variety of approaches has been proposed, only a few have reached the clinical arena. In particular, the most widely reported application of AM regards the use of FDM for cranio-maxillo-facial surgery, which involves the surgical treatment of congenital or acquired deformations for both functional and aesthetic purposes. Schantz et al. [135] used PCL scaffolds as burr hole plugs in a pilot study for cranioplasty. Implants were computer-designed with a fully interconnected honeycomb-like architecture fabricated by FDM. The clinical outcome after 12 months was positive, with all patients tolerating the implants with no adverse side-effects reported.

It is expected that future work in this field will demonstrate the potential of other AM methods – and hence other scaffold architectures – to enter the clinical arena [136].

Critical-sized defects have in many cases presented insurmountable challenges for bone tissue engineering. In this scenario, the incorporation of a functional vasculature able to meet mass transport requirements represents a major concern [137,138]. Innovative solutions are based on the inclusion of specific architectural features in scaffold design. Among others, there is the introduction of pre-designed multibranched vessel structures [84,139] and the fabrication of architectures mimicking the multidimensional hierarchy of native bones [10].

However, due to resolution limits, AM techniques at present are not suitable to directly manufacture hierarchical structures. To overcome this limitation, some authors have attempted to create hierarchical porous structures (with pore values spanning the macro to the sub-micron range) by combining AM techniques with polymer templating [140] or controlled sintering [141] approaches. The introduced sub-micron porosity has been demonstrated to promote vascularization and bone ingrowth, possibly due to cell–cell interactions occurring on that length scale. Alternatively, to overcome the difficulties arising from the incorporation of porogens or effusive solvents, indirect AM techniques [142] and other AM-related methods [143] have been investigated to obtain scaffolds with both locally and globally controlled porous inner architectures.

4.2. Cartilage

As previously underlined, another common application of AM techniques is the production of scaffolds for cartilage tissue engineering. Over the past two decades, different designs and manufacturing techniques have been investigated for developing TE scaffolds suitable for the fabrication of cartilage tissue substitutes, the most common being porous 3-D sponges and nonwoven fibrous structures [144]. However, a more homogeneous distribution of chondrocytes and ECM components as well as an enhanced differentiation both in vitro and in vivo have been detected in the case of AM scaffold architectures [145]. Control of design parameters (including total porosity value, pore size, geometry and distribution as well as pore accessibility and tortuosity) has been demonstrated to play a significant role in the morphology, composition, mechanical properties and functionality of the neo-cartilaginous tissue [144]. In this regard, low permeability scaffolds with spherical pore shapes have been demonstrated to better enhance the chondrogenic performance of chondrocytes in terms of matrix production and in vitro mRNA gene expression, with respect to a highly permeable scaffold with cubical pore shape [146]. Advances in fabrication technologies have enabled the strategic design of scaffolds with complex, biomimetic structures and properties. Some of the recent and most promising designs based on advanced fabrication techniques include the use of hybrid and/or "zonal" scaffolds to manufacture cartilage substitutes that more closely replicate the biomechanical characteristics and the stratified organization of native articular cartilage [144]. Composed of two essential components, hydrogel–solid hybrid scaffolds resemble the biphasic nature of articular cartilage and might be an effective strategy for TE cartilage repair. Indeed, in hybrid scaffolds, a solid polymer (usually a polyester) provides a reinforcing skeleton inferring mechanical strength, while the hydrogel phase represents a cell supportive/delivery matrix.

Alternatively, graded architectures with an orthogonal lay-down pattern have been fabricated by 3-D fiber deposition with the aim to obtain pore volume gradients that could be instructive for cell and/or ECM distribution. Results indicated that an anisotropic scaffold architecture could promote inhomogeneous tissue formation, but this was not enough to produce a zonal cartilage arrangement similar to natural tissue [145]. Application of depth-dependent mechanical cues within chondrocyte-seeded constructs has also been considered as an alternative approach to induce depth-varying inhomogeneity in material properties and hence in matrix content [147].

4.3. Osteochondral segment

Another popular research field regards the use of AM techniques in the regeneration of complex and multiple tissues, such as in osteochondral tissue engineering. In this case, bi-layered scaffolds have been developed with the aim to ideally promote individual growth of both cartilage and bone layers within a single integrated implant. Biphasic scaffolds (i.e. containing discrete regions that are optimized for selective growth of bone and cartilage) have been developed using different material types, internal architectures (e.g. porosity, pore interconnectivity), cells and biological factors, and in a limited number of cases they have reached the in vivo evaluation phase. In particular, biphasic scaffolds with a honeycomb 0/60/120° lay-down pattern produced by FDM have been considered for in vitro and in vivo tests. A homogeneous PCL scaffold with honeycomb structure was additively manufactured and in vitro tested by co-culturing osteoblasts and chondrocytes [148]. The study demonstrated that both cell types can proliferate, migrate and produce specific ECM in each scaffold compartment, providing a good integration at the interface. However, the maintenance of the required cellular phenotype is one of the critical points related to the use of different cell sources. Thus, stem/stromal cells (e.g. bone-marrow-derived mesenchymal stromal cells, BMSCs) have also been investigated for osteochondral tissue engineering [149]. Shao et al. [62] attempted to evaluate the repair potential of a hybrid PCL/PCL-tricalcium phosphate (cartilage and bone component, respectively) AM scaffold using BMSCs in a rabbit model. The proposed scaffold revealed superior repair

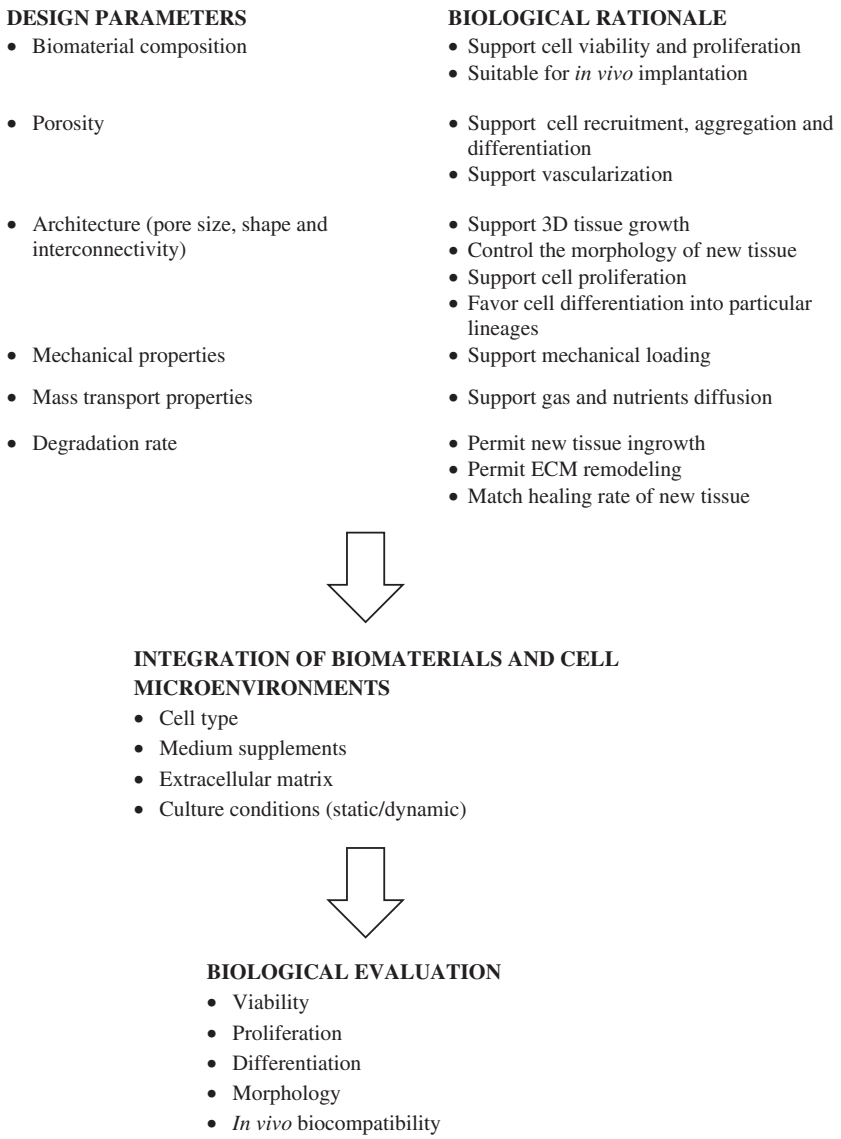


Fig. 5. Schematic representation of the requirements for a biologically informed scaffold design (modified from Refs. [131,132]).

ability in both bone and cartilage compartments. However, 6 months after implantation, although the regenerated segment showed good subchondral bone formation and a hyaline-like cartilage surface, cell arrangement in the newly formed cartilage lacked the typical zonal organization.

Alternatively, AM and/or indirect AM techniques have been successfully combined with other conventional scaffold fabrication approaches for developing bi-layered structures with additional features and functionalities. For example, Schek et al. [150] obtained bi-layered composite scaffolds using indirect solid free-form fabrication to produce molds for the fabrication of a hydroxyapatite bone compartment, which was coupled to a poly-L-lactide foamy cartilage section obtained by particulate leaching. Similarly, FDM and electrospun membranes have been combined to reproduce a biphasic scaffold for simultaneous regeneration of alveolar bone/periodontal ligament complexes [83].

Furthermore, to guarantee a functional interface between bone and cartilage, a multilayer osteochondral scaffold has been fabricated and in vitro evaluated in combination with BMSCs [151]. This bio-inspired scaffold was fabricated using a combination of stereolithography and gel-casting techniques, and included a bone phase with a 3-D channel network, a cartilage phase and a transitional structure that was designed according to the biological transitional interface from cartilage to bone.

Finally, preliminary studies have been devoted to assess the suitability of 3DF to fabricate cell-laden, heterogeneous hydrogel constructs for potential use as osteochondral grafts [152]. Although the obtained results are encouraging, poor mechanical properties and insufficient interaction between cells of adjacent layers are among the major limitations to the applicability of this approach.

Despite the variety of materials, designs and cell sources that have been investigated for AM-based osteochondral tissue engineering, an optimal strategy has not yet been identified, and novel combinations of materials and methodologies are needed before they can be applied in clinical practice.

4.4. Soft tissues

Targeting the regeneration of soft tissues and organs by AM, a popular trend consists in scaffold-less approaches, in which "bioinks" containing cell suspensions are deposited onto dense hydrogel supporting materials ("biopaper") [153]. However, given the

focus of the present review, only scaffold-based approaches will be considered in the following. Among the variety of AM scaffolds explored for soft tissue regeneration, a small minority is represented by solid 3-D microstructures obtained by processing thermoplastic biopolymers. For example, different microarchitectures have been fabricated and evaluated in vitro for cardiac tissue engineering purposes [128,154]. AM structures have also been used as a reinforcing framework in combination with other scaffold fabrication techniques for soft tissue applications, such as tissue-engineered vascular grafts [155]. However, thermoplastic polymers tend to be stiffer than native soft tissues, they degrade by bulk hydrolysis and fail under long-term cyclic loading [156].

For this reason, hydrogels represent another popular class of materials to mimic the mechanical and biological properties of soft tissues. Due to the intrinsic low mechanical properties of hydrogels, however, the generation of clinically relevant structures has been mainly addressed by the fabrication of non-self-supporting networks that do not exhibit external geometric complexity [157]. Furthermore, to obtain an accurate reproduction of the designed architecture, hydrogels have to meet specific requirements in terms of viscosity and gelation rate, which limit the number of formulations that can be applied to AM [2].

Recent findings have evidenced the difficulty in replicating complex organs by seeding pre-fabricated 3-D scaffolds [158]. Hence, direct manufacture of tissue precursors with a cell density similar to native tissue might represent a solution to overcome extensive in vitro culture. In this context, hydrogels represent an interesting starting material for cell encapsulation and delivery, and this has stimulated increasing research efforts to overcome their limited processability. As some AM processes involving hydrogels operate at room temperature, hydrogel scaffolds containing cells and growth factors have been successfully fabricated without affecting cell viability. Several research groups have adapted different AM techniques to assemble living cell-laden constructs directly from computer-generated design models with high resolution, aiming to demonstrate their abilities in the area of complex tissue and organ manufacturing [158]. The ability to control the position of many cell types, extracellular matrices and growth factors at different length scales is strictly required for this kind of application. Consequently, cell–hydrogel constructs have been investigated for the regeneration of tissues with complex, multilayered architectures, such as skin [159]. At present, however, these constructs fail in accurately recapitulating the nanoscale features of ECM components [160].

5. Conclusions and future directions

As demonstrated in the previous sections, scaffold design criteria have a significant impact on the overall tissue regeneration outcome, and optimization strategies are needed to compromise between often conflicting requirements.

Following the diffusion of CATE, the number of strategies pursuing an optimal scaffold design for a specific clinical application has tremendously increased. By integrating empirical, laboratory, computer modeling and simulation-based activities, CATE has eased the advance of scaffold-based TE to a multidisciplinary research field. In particular, the combination of experimental analyses and numerical simulations has resulted in a more efficient procedure to develop scaffolds addressing target requirements and has facilitated the exploration of several novel design principles incorporating biomimetic and biological features into the same architecture.

Although some general trends can be drawn, significant research effort is still necessary prior to understanding all the specifications for biologically informed scaffold design. In the last couple of years, only a few of the developed design criteria have been implemented in scaffold production. In addition, results of extensive in vitro and in vivo characterization are limited to a restrict number of tissues and architectures. Space-filling curves, with orthogonal or honeycomb patterns, together with image-based designs, are among the most widely employed and characterized methods. On the contrary, irregular porous structures, although seeming to better match native tissue complexity, have not been exhaustively validated. Implementation of TPMS design has recently provided encouraging preliminary results, but further work is needed to demonstrate the full potential of such methodology in in vivo settings.

Additionally, a significant gap in advancement between hard and soft tissue applications has to be pointed out. Indeed, the most significant progresses in CATE are expected for soft tissue engineering and organ regeneration. For these applications, novel CAD models and design methods need to be extensively explored in the near future. In this regard, hybrid scaffolds that integrate the advantages of several materials in the same microstructure may exhibit better properties targeting the reconstruction of multi-tissue or complex organs. To date, however, few data regarding rapid prototyping of multimaterial scaffolds are available, and even less are the works integrating materials selection in the FEA prediction of scaffold microarchitectural features.

Another fascinating trend to be reported concerns the integration of AM techniques with other scaffold fabrication methods to obtain hybrid architectures with complementary structural features. Although this innovative approach is still at its beginning, it is starting to give positive results not only in the recapitulation of complex tissues, but also in the enhancement of biological processes such as constructs vascularization.

Disclosures

The authors declare no conflict of interest.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 3 and 4, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi: 10.1016/j.actbio.2013.10.024.

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