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1 **Rhombicuboctahedron Unit Cell Based Scaffolds for Bone**
2 **Regeneration: Geometry Optimization with a**
3 **Mechanobiology – driven Algorithm**

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1 **ABSTRACT**

2 In a context more and more oriented towards customized medical solutions, we propose a
3 mechanobiology-driven algorithm to determine the optimal geometry of scaffolds for bone
4 regeneration that is the most suited to specific boundary and loading conditions. In spite of the huge
5 number of articles investigating different unit cells for porous biomaterials, no studies are reported in
6 the literature that optimize the geometric parameters of such unit cells based on mechanobiological
7 criteria. Parametric finite element models of scaffolds with rhombicuboctahedron unit cell were
8 developed and incorporated into an optimization algorithm that combines them with a computational
9 mechanobiological model. The algorithm perturbs iteratively the geometry of the unit cell until the
10 best scaffold geometry is identified, i.e. the geometry that allows to maximize the formation of bone.
11 Performances of scaffolds with rhombicuboctahedron unit cell were compared with those of other
12 scaffolds with hexahedron unit cells. We found that scaffolds with rhombicuboctahedron unit cell are
13 particularly suited for supporting medium-low loads, while, for higher loads, scaffolds with
14 hexahedron unit cells are preferable. The proposed algorithm can guide the orthopaedic/surgeon in
15 the choice of the best scaffold to be implanted in a patient-specific anatomic region.

16 *Keywords:* Computational Mechanobiology; Morphology Optimization; Scaffold Unit Cell;
17 Rhombicuboctahedron.

18

1. Introduction

Design of scaffolds for bone tissue engineering is a very challenging task that requires multi-disciplinary and multi-scale approaches [1,2]. Recent researches demonstrated that geometry and elastic properties of porous scaffolds significantly influence the cellular response and the rate of bone tissue regeneration process [3,4]. However, to date, a deep knowledge of the complex biological and biophysical phenomena that take place during the bone regeneration and that clearly explains how the scaffold geometry affects this process, is missing. The identification of all the geometrical features that might have an influence on bone tissue regeneration requires the systematic study of different classes of pore shapes but no such studies are currently available in the literature [3]. Very often, in order to partially bridge this information gap, scaffold designers utilize computational mechanobiological models that, combined with *in vivo* and *in vitro* experiments allow to gain a deeper understanding of the possible relationships existing between the cellular process of mechano-transduction, the mechanical environment and the scaffold geometry [5-11]. In other words, computational mechanobiological models are often utilized to determine the quantitative rules that govern the effects of mechanical and geometrical factors on tissue differentiation and bone regeneration in bone tissue scaffolds [12]. A number of recent papers using multi-physics modelling have investigated the effects of biophysical stimuli on bone cells in scaffolds. Jungreuthmayer *et al.* [13] quantified the deformation of cells seeded on a collagen-GAG scaffold which was perfused by culture medium inside a flow perfusion bioreactor. McCoy *et al.*, [14] examined how the scaffold mean pore size influences cell attachment morphology and subsequently impacts upon cell deformation and detachment when subjected to fluid-flow. Zhao *et al.* [15] determined the mechanical stimulation of osteoblasts seeded in a biomaterial scaffold under flow perfusion and mechanical compression using multiscale modelling by twoway fluid–structure interaction and FE approaches. Zhao *et al.* [16] applied a fluid–structure interaction model to investigate the role of scaffold geometry

1 (architecture, pore size and porosity) on pore wall shear stress under a range of different loading
2 scenarios. Other authors predicted and measured in vivo stress and strain stimuli [17-21].

3 Following the recent development of more and more sophisticated rapid prototyping techniques
4 that allow the fabrication of solids with arbitrarily complex geometry, an increasing attention was
5 devoted to the design of unit cell geometries for regular porous biomaterials [22]. Relationships
6 between scaffold micro-architecture and resulting mechanical and biological properties were
7 extensively investigated in a number of articles [23-30]. Different unit cell geometries were studied
8 such as cubic, rhombic dodecahedron, pyramidal and diamond and for some of them an analytical
9 solution was derived that expresses the apparent mechanical properties in function of the unit cell
10 geometric parameters. For instance, based on the Euler-Bernoulli beam theory, Hedayati *et al.*
11 developed the analytical solutions that relate the dimensions of the repeating unit cell to the elastic
12 modulus, Poisson's ratio and yield stress of porous biomaterials based on the rhombicuboctahedron
13 [31] and the truncated cube [32] unit cells. Despite these studies, our understanding of
14 mechanobiology of tissue differentiation in scaffolds remains still incomplete and to the knowledge
15 of the present authors, no studies are reported in the literature that optimize the geometric parameters
16 of such unit cells based on mechanobiological criteria. The optimization of the geometry of bone
17 tissue scaffolds is the objective of a number of studies [33]. Most of them identified the best scaffold
18 geometry based on criteria other than the mechanobiological ones [34-38]. In previous studies, we
19 developed a mechanobiology-based optimization algorithm to determine the best scaffold geometry
20 with both, constant [39] and functionally graded [40] porosity. The scaffolds investigated in these
21 studies included a simple hexahedron unit cell obtained as a cubic volume with three holes generated,
22 each, as the cut-protrusion of rectangular (or, in the simpler case, square) or elliptic (or, in the simpler
23 case, circular) contours along the three orthogonal directions of the coordinate axes. In the present
24 study, we further developed this model by including a much more complex and sophisticated unit
25 cell, i.e. the rhombicuboctahedron unit cell. The recent interest devoted to this relatively unexplored
26 unit cell [31] led us to investigate its mechanobiological behaviour as well as to compare its

1 performances with those of hexahedron unit cell. The beam elements of which the
2 rhombicuboctahedron unit cell is comprised were hypothesized to possess both, square and circular
3 cross-section. In detail, the proposed algorithm combines a computational mechanobiological model,
4 a structural optimization routine and the Finite Element (FE) model of a scaffold subjected to
5 compression load. The algorithm perturbs iteratively the geometry of the unit cell and hence of the
6 entire scaffold model until the best scaffold geometry is identified, i.e. the geometry that allows
7 maximizing the amounts of bone predicted to form. The optimal scaffold geometry was determined
8 for different levels of the: (i) scaffold Young's modulus, (ii) load acting on the scaffold and (iii) length
9 of each beam element included in the unit cell.

10 **2. Materials and Methods**

11 *2.1. CAD models of the rhombicuboctahedron unit cell and of the entire scaffold*

12 The model of the unit cell successively discretized into finite elements, was first generated in
13 CAD environment. Both, the CAD and finite element models were generated through the general
14 purpose software ABAQUS® Version 6.12 (Dassault Systèmes, France). In detail, the generation of
15 CAD was performed by adopting a seven-step procedure. In the first step (Fig. 1(a)), an octagonal
16 trajectory t_{oct} was traced, a circular or square cross-section was created and then protruded, by means
17 of the tool 'sweep' available in ABAQUS, along t_{oct} . All the sides of t_{oct} trajectory were hypothesized
18 to have the same length L . The same procedure described for step 1 was successively repeated in steps
19 2 to 6, (Figs 1(b) to 1(f)) adding a solid volume - protruded along octagonal trajectories t_{oct} - in each
20 step. In step 7 (Fig. 1(g)) the solid so obtained was cut along planes π_{Mi} ($i=1, 2, \dots, 6$) delimiting the
21 cubic volume $Q \times Q \times Q$ containing the unit cell. Dimension Q is related to the length L of each side
22 of t_{oct} through the relationship:
23

$$Q = L \cdot \left(1 + 2 \cdot \cos\left(\frac{\pi}{4}\right)\right) \quad (1)$$

1

3

4 Protrusions were carried out with a circular (Fig. 1(h)) or a square (Fig. 1(m)) cross-section. Three
5 different values of L were hypothesized: $L = L_0 = 263.85 \mu\text{m}$, $L = L_- = L_0 \times 0.9$ and $L = L_+ = L_0 \times 1.1$.
6 With these values, the resulting scaffold dimensions are consistent with those hypothesized in
7 previous studies [39,40]. The cross-section dimension D , i.e. the diameter of the circular cross-section
8 (Fig. 1(h)) and/or the side length of the square cross-section (Fig. 1(m)) was fixed as a parametric
9 variable and its value was optimized via the algorithm described below.

10 By successively mirroring the unit cell with respect to planes π_{Mi} the entire scaffold geometry
11 including $4 \times 4 \times 4 = 64$ unit cells was obtained (Fig. 2). According to previous studies [5] the volume
12 enclosed within the scaffold structure, - i.e. the volume of the scaffold pores, - was hypothesized to
13 be occupied by granulation tissue. The CAD model of this tissue (represented in red, Fig. 2(b)) was
14 generated through the boolean operation of subtraction (from the cubic volume $4Q \times 4Q \times 4Q$, the
15 volume occupied by the scaffold itself). The resulting scaffold system (scaffold + granulation tissue)
16 had dimensions $h \times h \times h$ (Fig. 2(c)), where h is a function of L . In particular, in the case where $L =$
17 L_0 , it was $h = h_0 = 4 \times Q = 4 \times L_0 \times (1 + 2 \times \cos(\pi/4)) = 4 \times 637 = 2548 \mu\text{m}$; analogously, in the case
18 $L = L_-$, it was $h = h_0 \times 0.9 = 2293.2 \mu\text{m}$; finally, in the case $L = L_+$, it was $h = 1.1 \times h_0 = 2802.8 \mu\text{m}$.
19 Values of h investigated in this study are consistent with those hypothesized in previous articles
20 [39,40].

21 Exploiting the symmetry of the system with respect to the planes π_{1-2} and π_{3-2} (Fig. 2(c)), only
22 1/4 of the entire model was studied that included $2 \times 2 \times 4 = 16$ unit cells for a total volume $V_{TOT} =$
23 $h/2 \times h/2 \times h$ (Fig. 2(d)).

24 2.2. Finite element modelling of scaffolds subjected to compression loading

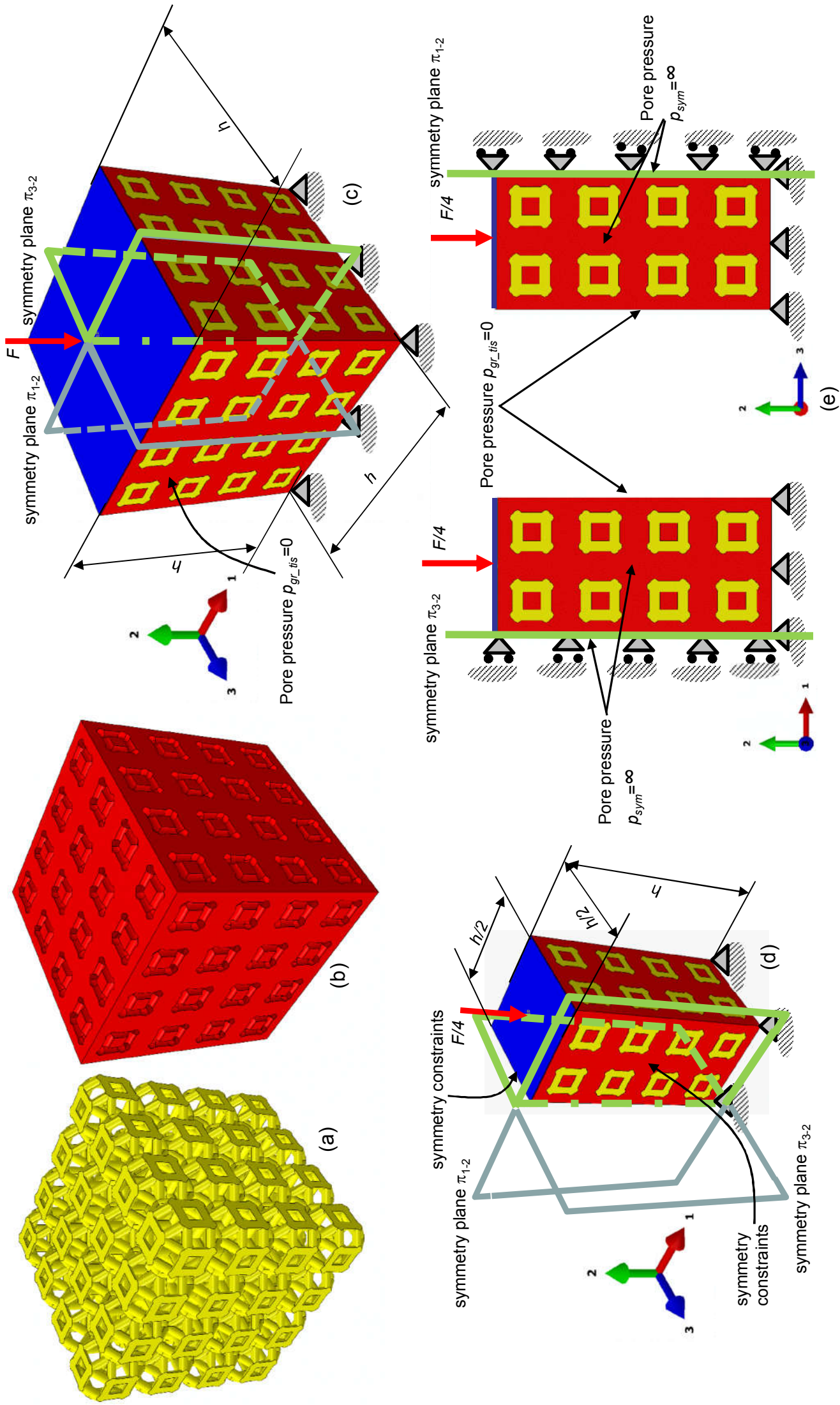


Fig. 2. Boundary and loading conditions acting on the scaffold model. CAD model of the scaffold (a), granulation tissue (b) and entire (including 64 unit cells) system (scaffold + granulation tissue) (c). Exploiting the symmetry of the problem, a simplified model reproducing only 1/4 of the entire geometry was considered (d). (e) Detailed views of the simplified model with indicated the boundary and loading conditions acting on it. As the surrounding tissue would not totally prevent the fluid from escaping, the pore pressure p_{gr_tis} on the outer surfaces of the granulation tissue was set equal to zero to simulate the free exudation of fluid. On the symmetry planes instead, being null the fluid flow, the pore pressure must be $p_{sym} = \infty$.

1 The CAD models with both, circular and square cross-sections were discretized into finite elements
2 (Fig. 3). Models of scaffold and granulation tissue were hypothesized to be made from poroelastic
3 materials and discretized with tetrahedral four-node, linear displacement and pore pressure elements
4 (C3D4P, see ABAQUS Documentation). Setting an average element dimension of 20 μm , the model
5 (i.e. scaffold + granulation tissue) included about 5×10^6 elements with about 1×10^6 nodes.

6 The scaffold model was hypothesized to be subjected to compression loading. To this purpose,
7 the nodes of the bottom surface were clamped (Figs. 2(c) to 2(e)) while a compression load was
8 applied on the scaffold upper surface via a rigid plate (represented in blue, Fig. 2). Constraint
9 equations were utilized between the rigid plate (RP) and the scaffold upper surface (SUS) to impose
10 the equality of the displacement fields u_2 of the two surfaces: $u_2(\text{RP}) = u_2(\text{SUS})$ (Fig. 2(c)). The
11 concentrated force F acting on the plate produces a uniformly distributed load p given by: $p = F / (h$
12 $\times h)$, being $h \times h$ the area of the plate surface. F was hypothesized to assume five different values
13 corresponding to the pressures p : 0.05, 0.1, 0.5, 1.0 and 1.5 MPa which are consistent with the
14 pressure values hypothesized by Byrne *et al.* [5]. The load, according to previous articles [39,41],
15 was applied following a linear ramp in the time interval of 1 second that is the hypothetical time in
16 which a scaffold can be loaded in physiological conditions. The hypothesized values of pressure
17 correspond to medium-low values of load acting on the spongy bone, which shows an ultimate
18 strength in compression of 10÷20 MPa depending on its apparent density [42]. According to Byrne
19 *et al.* [5], the pore pressure p_{gr_tis} acting on the outer surface of the granulation tissue was imposed to
20 be nil, (Figs. 2(c) and 2(e)) which means that during the compression the fluid can freely exudate.
21 Symmetry constraints were applied on the surfaces of the model lying on the symmetry planes π_{1-2}
22 and π_{3-2} (Figs. 2(d) and 2(e)). In particular, an infinite pore pressure ($p_{sym} = \infty$) was applied on these
23 surfaces which indicates that fluid flow is not allowed.

24 Regarding the mechanical properties implemented in the FE model, we hypothesized three
25 different values of the scaffold Young's modulus: 500, 1000 and 1500 MPa, which are consistent
26 with those utilized by Byrne *et al.* [5]. The same material properties were also utilized in previous

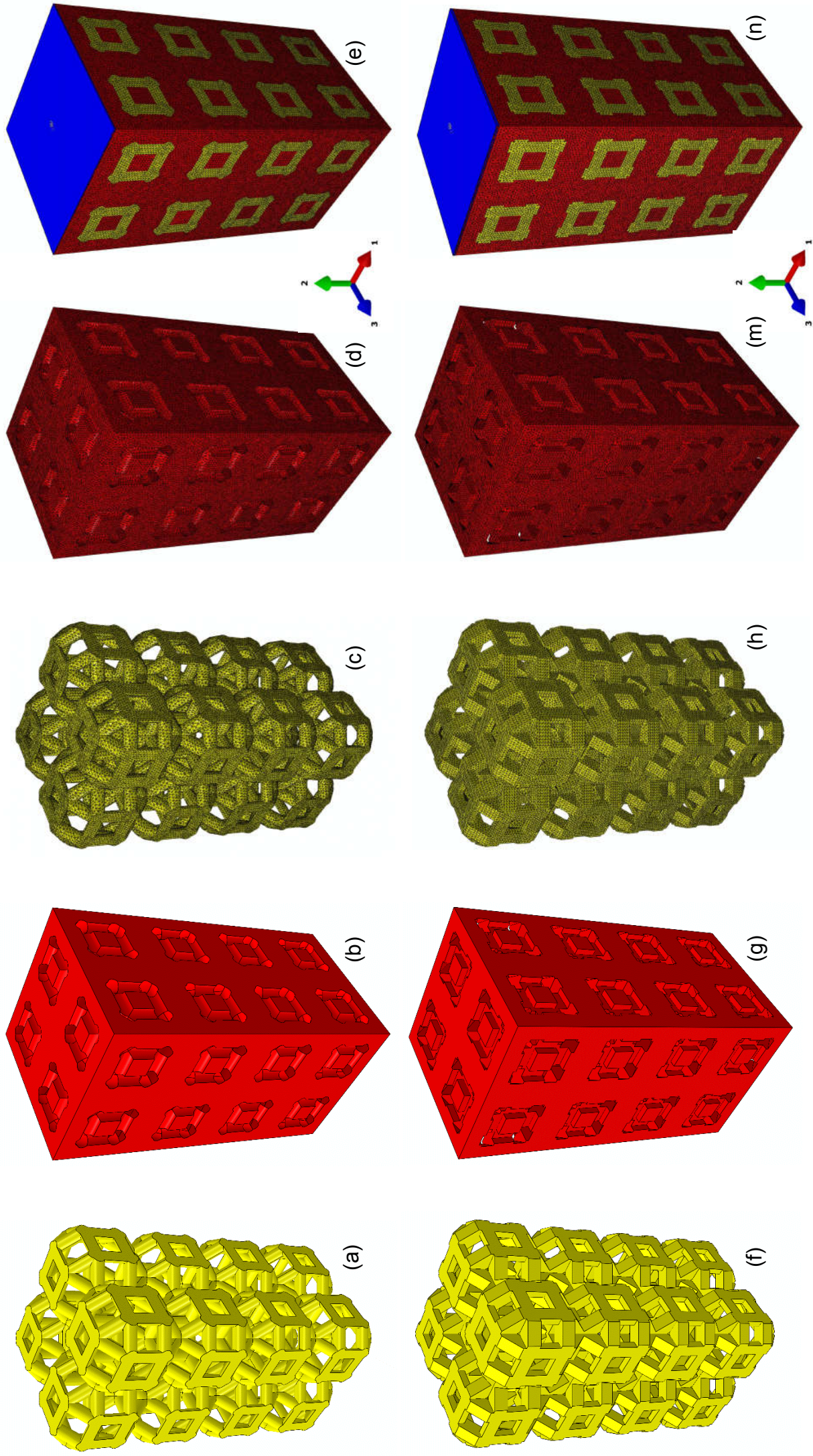


Fig. 3. CAD model of the scaffold (a, f) and of the granulation tissue (b, g) in the case of circular (a, b) and square (f, g) cross-section. Finite element mesh of the scaffold (c, h), the granulation tissue (d, m) and of the entire system (e, n) in the case of circular (c, d, e) and square (h, m, n) cross-section. The entire model (scaffold + granulation tissue) included about 5×10^6 elements with about 1×10^6 nodes.

1 studies [39,40]. An example of a real material used in fabricating scaffolds by selective laser sintering
2 and possessing a Young's modulus reasonably close ($E \approx 500$ MPa) to those hypothesized in this
3 study, is represented by the Polycaprolactone [43]. The other material properties implemented for the
4 scaffold model are the following [5]: permeability, $1 \times 10^{-14} \text{m}^4/\text{N/s}$; porosity, 0.5; Poisson's ratio, 0.3;
5 bulk modulus grain, 13920 MPa; bulk modulus fluid, 2300 MPa. Material properties implemented
6 for the granulation tissue are the same as those utilized in other articles [44-46]: Young's modulus,
7 0.2 MPa; permeability $1 \times 10^{-14} \text{m}^4/\text{N/s}$; Poisson's ratio, 0.167; porosity, 0.8; bulk modulus grain, 2300
8 MPa; bulk modulus fluid, 2300 MPa.

9 Nonlinear finite element analyses were performed that account for geometric nonlinearities.

10 2.3. Mechanobiological model based on deviatoric strain and fluid velocity

11 Once the Mesenchymal Stem Cells (MSCs) invade the scaffold and occupy its pores, their
12 differentiation begins thus triggering the tissue regeneration process. Following the hypothesis that
13 the transient phase of the cells dispersal has already terminated we assume that MSCs are
14 homogeneously distributed throughout the scaffold pores. Following the mechanobiological model
15 of Prendergast *et al.* [47] and according to Byrne *et al.* [5], we modelled the domain where
16 regeneration takes place as a poroelastic material and assumed the tissue differentiation process to
17 depend on a biophysical stimulus S that is, in turn, a function of the octahedral shear strain γ and of
18 the relative fluid/solid velocity v . In detail, if ε_I , ε_{II} and ε_{III} are the principal strains, and a and b the
19 two empirical constants defined by Huiskes *et al.* [48] as $a = 3.75\%$ and $b = 3 \mu\text{ms}^{-1}$, the octahedral
20 shear strain γ and the biophysical stimulus S are given by:

$$\begin{cases} \gamma = \frac{1}{2} \sqrt{(\varepsilon_I - \varepsilon_{II})^2 + (\varepsilon_{II} - \varepsilon_{III})^2 + (\varepsilon_I - \varepsilon_{III})^2} \\ S = \frac{\gamma}{a} + \frac{v}{b} \end{cases} \quad (2)$$

22

1 Based on the values of S , the model predicts formation of different tissue types. The following
 2 inequalities describe the tissue type corresponding to all the possible values S can assume:

3

$$\begin{cases}
 \text{if } S > c \text{ then fibrogenesis} \rightarrow \text{formation of fibrous tissue} \\
 \text{if } 1 < S < c \text{ then chondrogenesis} \rightarrow \text{formation of cartilage} \\
 \text{if } n_{mature} < S < 1 \text{ then osteogenesis} \rightarrow \text{formation of immature bone} \\
 \text{if } n_{resorb} < S < n_{mature} \text{ then osteogenesis} \rightarrow \text{formation of mature bone} \\
 \text{if } 0 < S < n_{resorb} \text{ then osteoclastogenesis} \rightarrow \text{bone resorbtion}
 \end{cases} \quad (3)$$

5

6 where n_{resorb} , n_{mature} , and m represent boundaries of the mechano-regulation diagram for tissue

7 differentiation. Their values were taken to be the same as those utilized by Lacroix and Prendergast

8 [49] ($n_{resorb} = 0.01$, $n_{mature} = 0.53$, $m = 3$). By utilizing the FE model above described, the strain and

9 the fluid velocity values were computed within the volume of the scaffold pores (represented in red,

10 Fig. 2(b)) and implemented into equations (2). Therefore, based on the values of S and according to

11 inequalities (3) the tissue type for each element was predicted. The specific succession of all the

12 operations followed to do this computation as well as the criterion utilized to determine the best

13 scaffold geometry are described in the following section.

14 2.4. The optimization algorithm implemented to determine the best scaffold geometry

15 An *ad hoc* algorithm was written in Matlab® (Version R2015b) environment integrating: (a) CAD

16 and FE scaffold models, (b) the mechano-regulation model described above and (c) a globally

17 convergent gradient-based structural optimization routine. A graphical summary of this algorithm is

18 shown in Fig. 4. The objective of the algorithm is to determine the best value of the cross-section

19 dimension D (for different cross-section shapes and for different values of the: (i) scaffold Young's

20 modulus E , (ii) length of the beam elements L and (iii) compressive load p) that allows to maximize

21 the formation of bone. The algorithm first requires to select: (i) the shape (Block [1]) of the cross-

22 section (circular or square, Block [2]); (ii) one of the three possible values (Block [3] and Block [4])

23 of the scaffold Young's modulus (i.e. $E = 500$, 1000 or 1500 MPa); (iii) one of the three possible

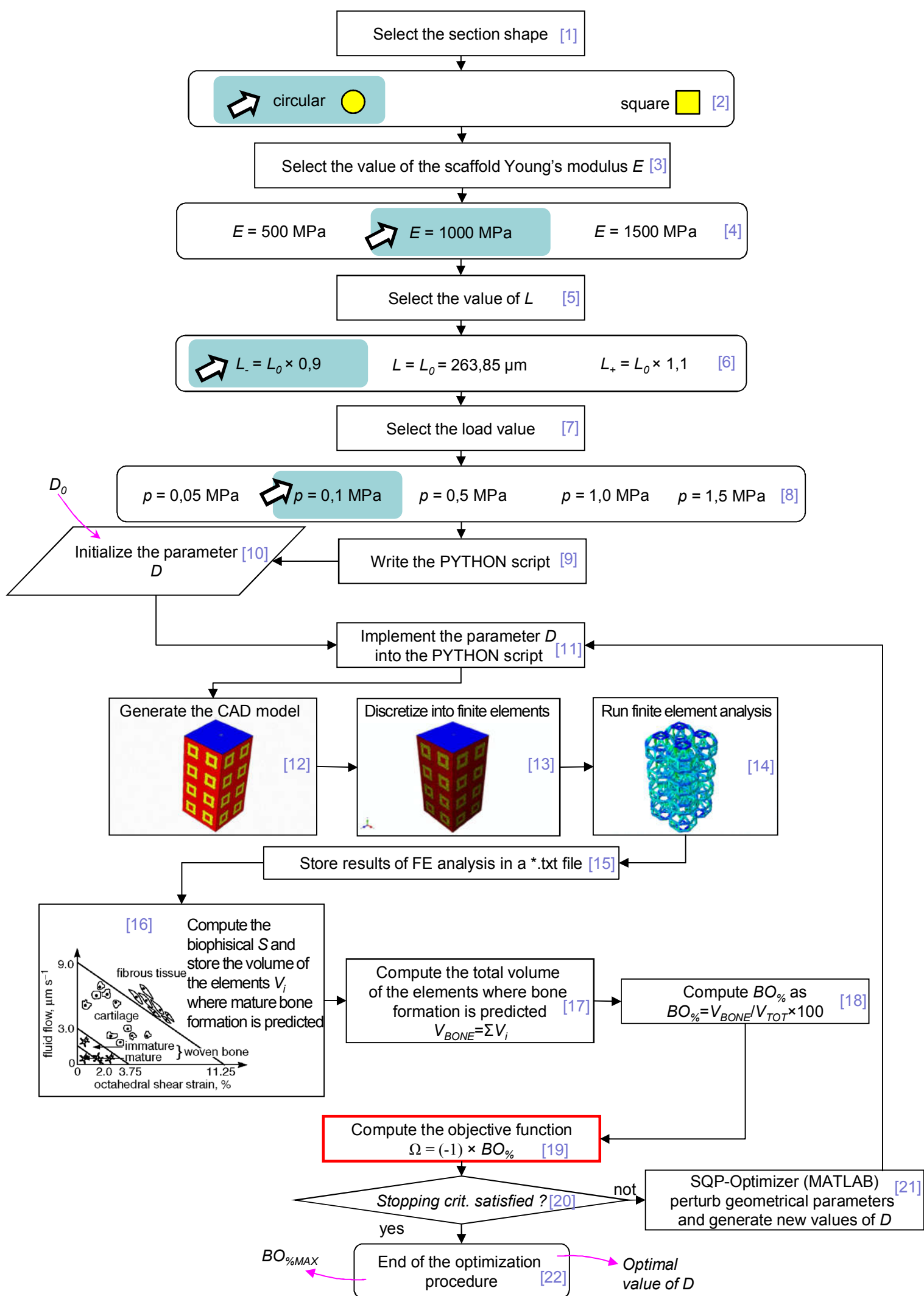


Fig. 4. Schematic of the algorithm written in Matlab environment to determine the optimal dimension D for different values of load, Young's modulus and L and for different cross-section shapes.

1 values of L (Block [5]) (Fig. 1, $L = L_0$, $L = L_-$ and $L = L_+$ (Block[6])); (iv) one of the five possible
2 values of the load p (Block [7]) ($p = 0.05, 0.1, 0.5, 1.0$ and 1.5 MPa (Block [8])). Based on the choices
3 made by the user, the algorithm writes the corresponding PYTHON script (Block [9]) and after asking
4 the user to initialize the value of the cross-section dimension D (Block [10]) implements this value
5 into the script itself (Block [11]). Then, the PYTHON script is given in input to ABAQUS that builds
6 the CAD model (Block [12]), generates the finite element mesh (Block [13]) and runs the finite
7 element analysis (Block [14]) with the boundary and loading conditions described above. Results of
8 the FE analysis including the values of the principal strains and those of the relative fluid/solid
9 velocity are stored in a *.txt file (Block [15]). According to equations (2) and (3), the algorithm
10 computes, for all the elements occupying the scaffold pores (represented in red, Figs. 2 and 3), the
11 biophysical stimulus S (Block [16]). In particular, for those elements where the formation of mature
12 bone is predicted (i.e. for the elements for which the inequality $n_{resorb} < S < n_{mature}$ is satisfied) the
13 algorithm stores the volume V_i . Once computation of S is terminated for all the elements, the
14 algorithm calculates the volume V_{BONE} given by the sum of all the volumes V_i : $V_{BONE} = \Sigma V_i$, (Block
15 [17]). Known V_{BONE} , $BO\%$, the percentage of the total scaffold volume occupied by bone is computed
16 as (Block [18]): $BO\% = V_{BONE} / V_{TOT} \times 100$. At this point, the objective function Ω is defined as the
17 opposite of $BO\%$ (Block [19]) and an optimization problem is formulated, where the cross-section
18 dimension D is the design variable while Ω is the objective to minimize:

19

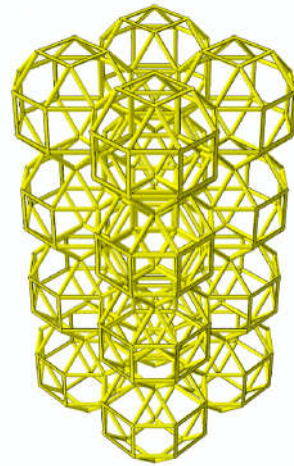
$$20 \quad \begin{cases} \text{Min} (\Omega (D) = (-1) \cdot BO\%) \\ D_{min} < D < D_{max} \end{cases} \quad (4)$$

21

22 D_{min} and D_{max} are the lower and the upper bounds, respectively, that were hypothesized to assume the
23 values listed in Fig. 5. In particular, regardless of the cross-section shape and of the value of L , D_{min}
24 was set equal to $28 \mu\text{m}$ (Figs. 5 (a) and 5 (b)). For the hypothesized values of load, in fact, cross-
25 section dimensions D smaller than $28 \mu\text{m}$ do not have any interest. Values of the upper bound D_{max} ,

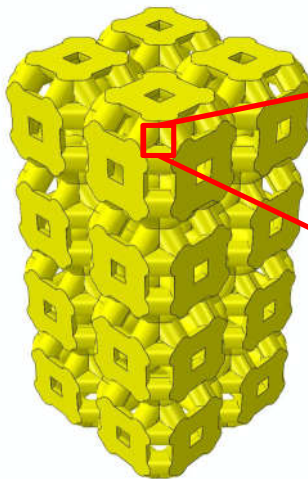
L	Section shape	D_{min} [μm]	D_{max} [μm]
L_0	circular	28.0	154.0
L_-	circular	28.0	142.0
L_+	circular	28.0	168.0
L_0	square	28.0	109.0
L_-	square	28.0	98.0
L_+	square	28.0	120.0

(a)



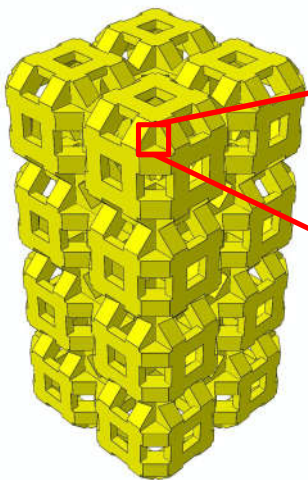
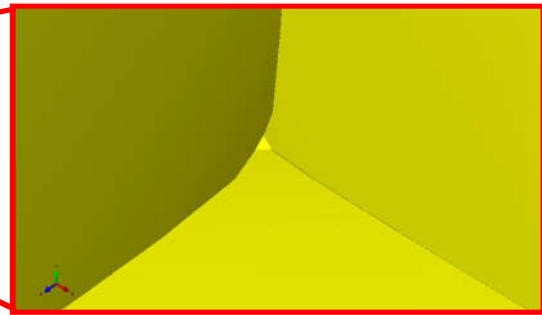
$$D = D_{min} = 28 \mu\text{m}$$

(b)



$$D = D_{max} = 154 \mu\text{m}$$

(c)



$$D = D_{max} = 109 \mu\text{m}$$

(d)

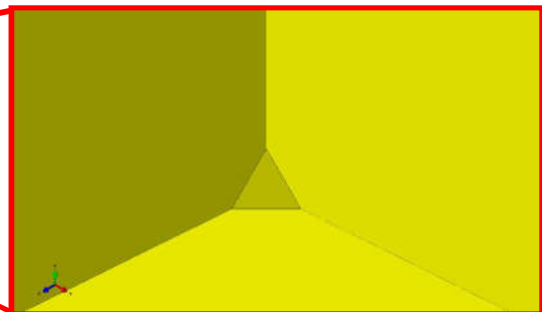


Fig. 5. (a) Upper and lower bounds set for the dimension D for different cross-section shapes and values of L . Geometric configuration of the model for $D = D_{min}$ (b) and $D = D_{max}$ in the case of circular (c) and square (d) cross-section. Values of $D > D_{max}$ lead to close the aperture between the inclined sides (see detailed images (c) and (d)) thus changing the originally hypothesized connectivity scaffold.

1 instead, were set in function of the cross-section shape and the beam length L . In general, values of
2 $D > D_{max}$ lead to close the aperture between the inclined sides of the triangular elements of the unit
3 cell (Figs. 5 (c) and 5 (d)) thus changing the originally hypothesized connectivity scaffold.

4 In order to solve the inverse problem described in equation (4), the optimization tool *fmincon*
5 available in Matlab® was utilized, that finds the minimum of a constrained nonlinear multivariable
6 function. The implementation of this solver requires the definition of stopping criteria that depend, in
7 turn, on the tolerance values fixed by the user. If one of these stopping criteria is met (Block [20],
8 Fig. 4), the algorithm stops and gives in output the optimal dimension of D and the corresponding
9 value of the percentage of the total scaffold volume occupied by bone $BO\%_{MAX}$ (Block [22]).
10 Conversely, if none of the stopping criteria is satisfied, the algorithm perturbs the scaffold geometry
11 generating new candidate solutions D (Block [21]). These are implemented into the PYTHON script
12 (Block [11]) that is given input to Abaqus thus commencing a new iteration cycle. It is worthy to note
13 that for each iteration cycle, a value of $BO\%$ is computed. Obviously, for how the objective function
14 is defined, the value of $BO\%$ computed in correspondence of the optimal dimension of D is the
15 maximum one (among all the other $BO\%$ computed in all the cycles performed) and is hence called
16 $BO\%_{MAX}$ (see Fig. 4, Block [22]).

17 Each optimization process included, on average, thirty iteration cycles, which means, in other
18 words, thirty finite element analyses to be run. Considering that each FE analysis took about 1.2 h, it
19 follows that an entire optimization process required approximately $30 \times 1.2 = 36$ h. Furthermore,
20 considering that in this study we investigated 2 cross-section shapes, three values of length L , three
21 values of the scaffold Young's modulus and five levels of compressive loads, it follows that a total
22 of: $2 \times 3 \times 5 \times 3 = 90$ optimization processes were carried out that took some 3200 h.

23 Computations were performed on a HP XW6600- Intel® Xeon® Dual-Processor E5-5450 3
24 GHz – 32 Gb RAM.

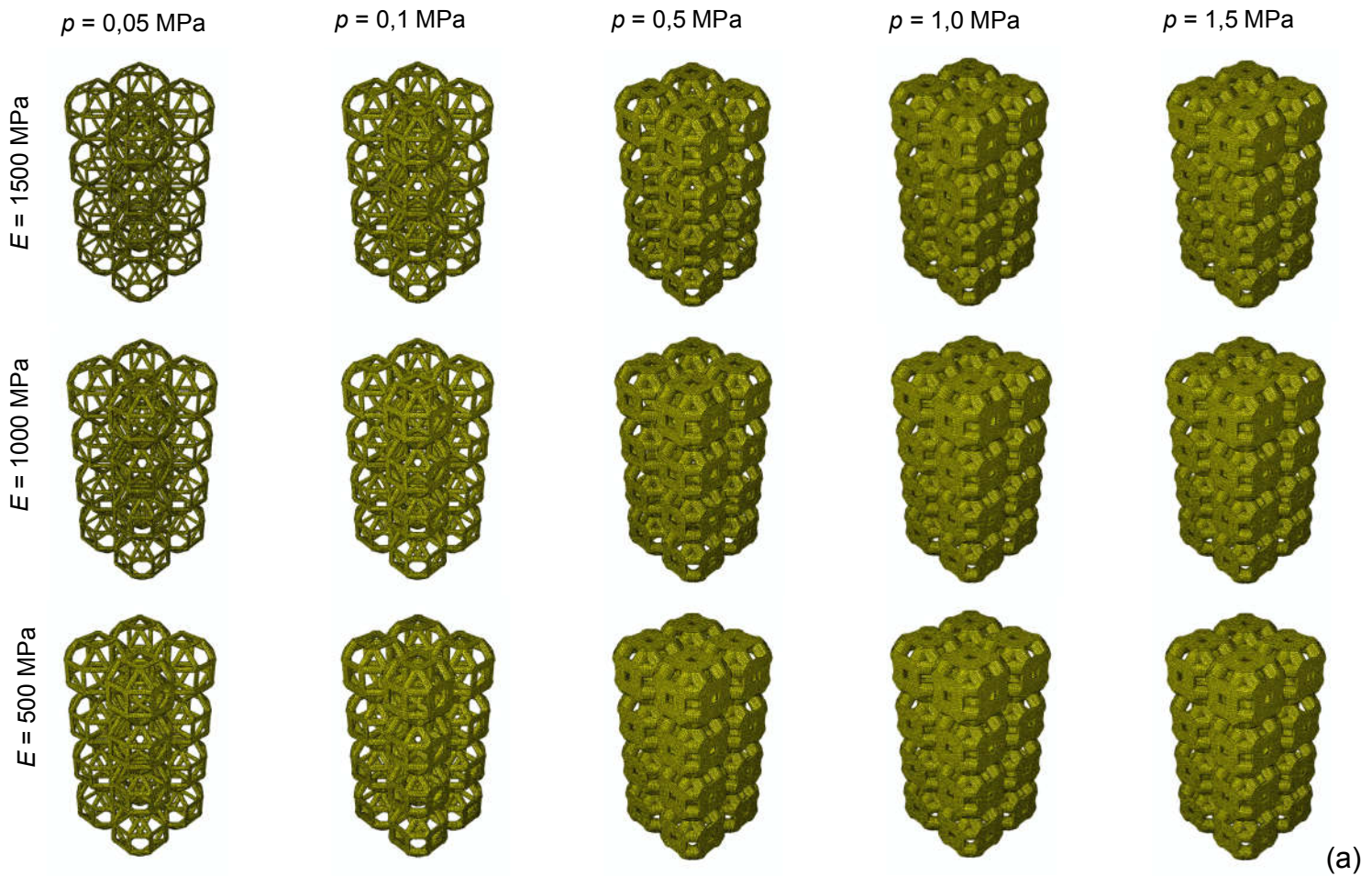
1 3. Results and Discussion

2 3.1. Effect of cross-section shape

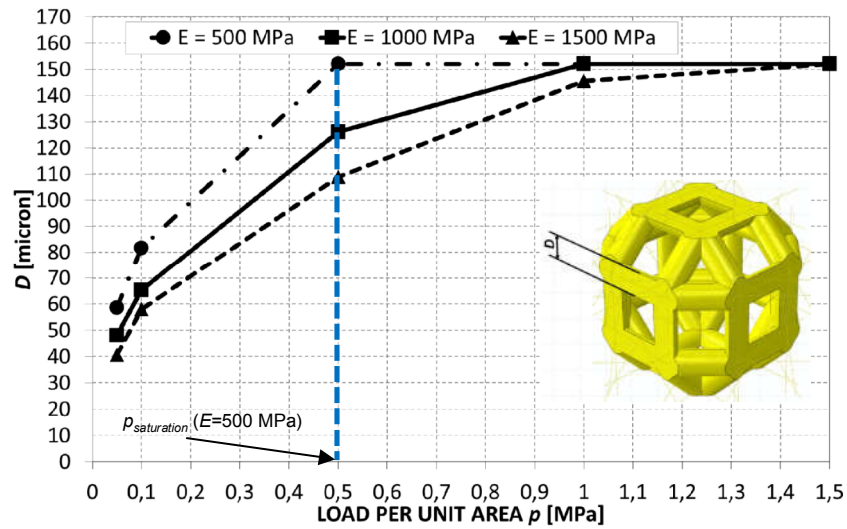
3 The optimal scaffold geometries predicted by the algorithm in the case of circular (Fig. 6(a))
4 and square (Fig. 7(a)) cross-section were consistent with our expectations. The optimal cross-section
5 dimension D increases for decreasing values of the scaffold Young's modulus E and for increasing
6 levels of the compressive load p . In fact, as E decreases, the global scaffold stiffness decreases too,
7 which implies higher strain values and hence larger formation of fibrous tissue. The algorithm, then,
8 tends to counterbalance this, increasing the cross-section dimension thus stiffening the scaffold. In
9 the same way, increasing values of p lead to larger deformations that lead, in turn, to higher levels of
10 the biophysical stimulus S and hence to fibrogenesis (see equation 3). The algorithm tends 'to move'
11 the average biophysical stimulus S towards the osteogenesis, by increasing D . Curves reporting the
12 optimal dimension D in function of the load (per unit area) p monotonically increase until the
13 asymptotic condition of saturation $D = D_{max}$ is reached (Figs. 6(b) and 6(c)). Once, for a load level,
14 the condition $p = p_{saturation}$ is reached, then the algorithm gives in output, for all the values of
15 $p > p_{saturation}$, the result $D = D_{max}$. As it can be seen (Figs. 6(b) and 7(b)), the saturation load $p_{saturation}$
16 depends on the scaffold Young's modulus and increases with it. Interestingly, $p_{saturation}$ values
17 computed for the circular cross-section are on average higher than those predicted for the square one.
18 In general, the higher the saturation load, the larger is the interval where the optimization algorithm
19 can find the optimal solution and hence, definitively, the larger the amounts of bone that can create
20 within the scaffold pores.

21 Increasing values of $BO_{\%MAX}$ are predicted for decreasing values of loads and for increasing
22 scaffold Young's moduli, in both cases, circular (Fig. 6(c)) and square (Fig. 7(c)) cross-section.

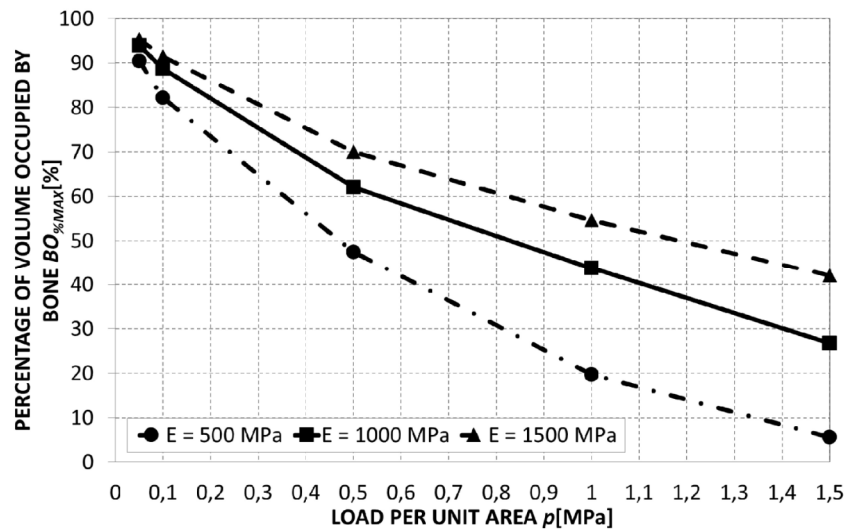
23 If V_{SCAFF} is the volume occupied by the scaffold, the percentage of volume occupied by the
24 scaffold is defined as $SCA\% = V_{SCAFF} / V_{TOT} \times 100$. Similarly, the percentage of volume occupied by



(a)

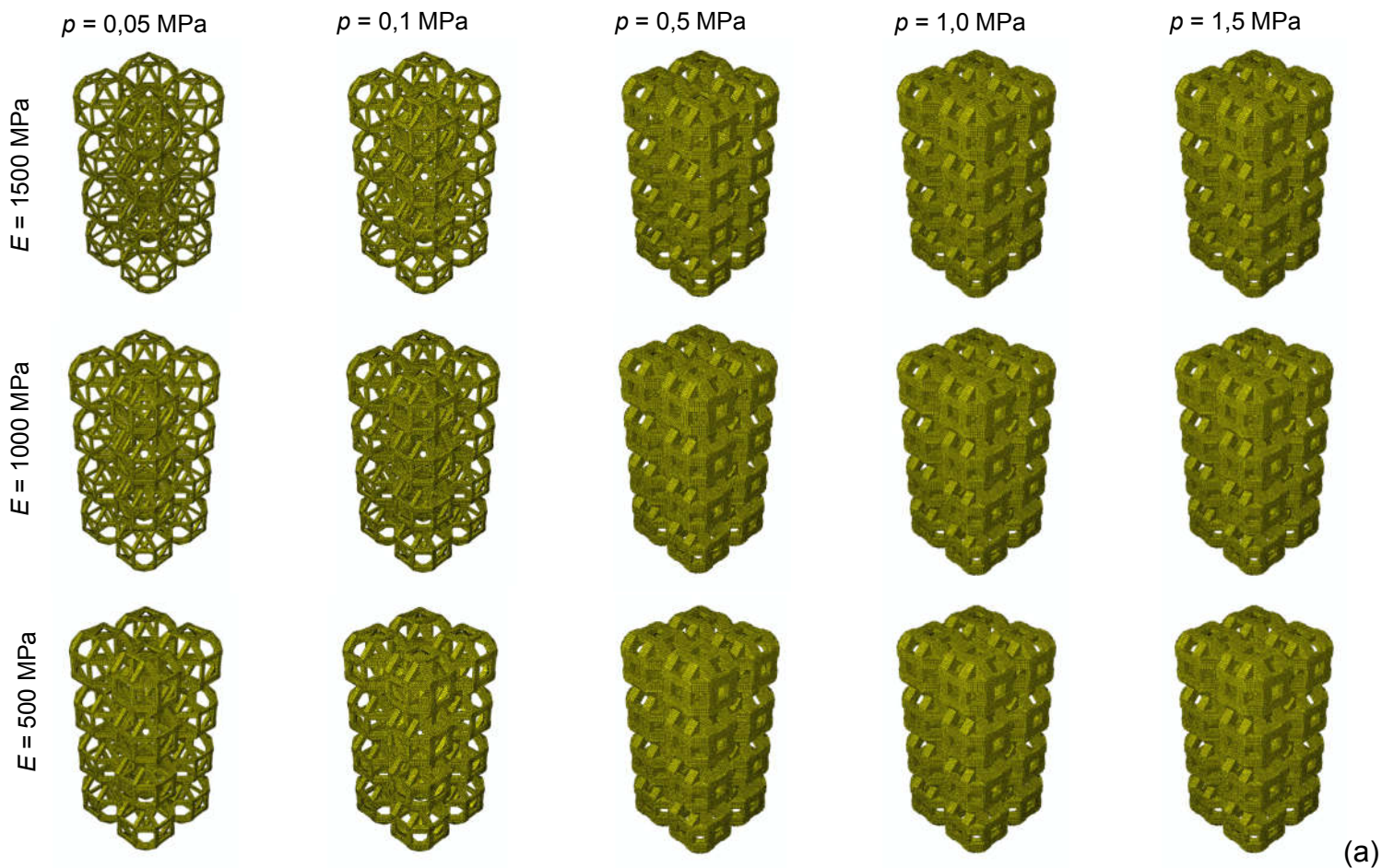


(b)

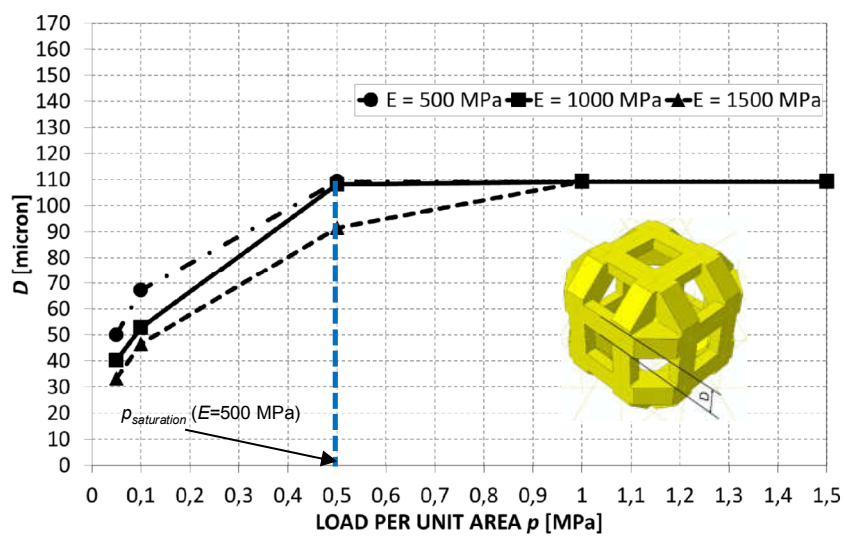


(c)

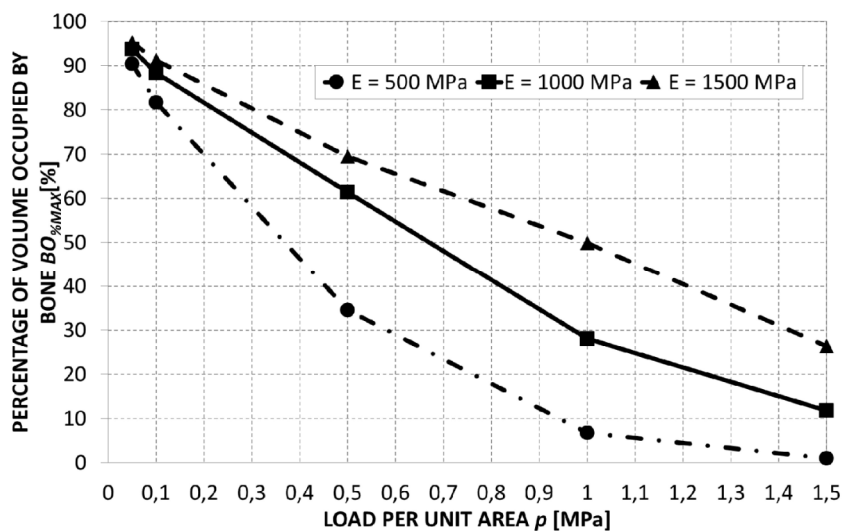
Fig. 6. Optimization of the rhombicuboctahedron unit cell, circular cross-section. (a) Optimal scaffold geometries predicted by the algorithm, in the case of circular cross-section. Values of the optimal diameter D (b) and of $BO_{\%MAX}$ (c) predicted by the algorithm for different levels of load and scaffold Young's modulus. For the sake of clarity, the only saturation load $p_{saturation}$ related to $E = 500$ MPa is shown in the figure.



(a)



(b)



(c)

Fig. 7. Optimization of the rhombicuboctahedron unit cell, square cross-section. (a) Optimal scaffold geometries predicted by the algorithm, in the case of square cross-section. Values of the optimal diameter D (b) and of $BO_{\%MAX}$ (c) predicted by the algorithm for different levels of load and scaffold Young's modulus. For the sake of clarity, the only saturation load $p_{saturation}$ related to $E = 500$ MPa is shown in the figure.

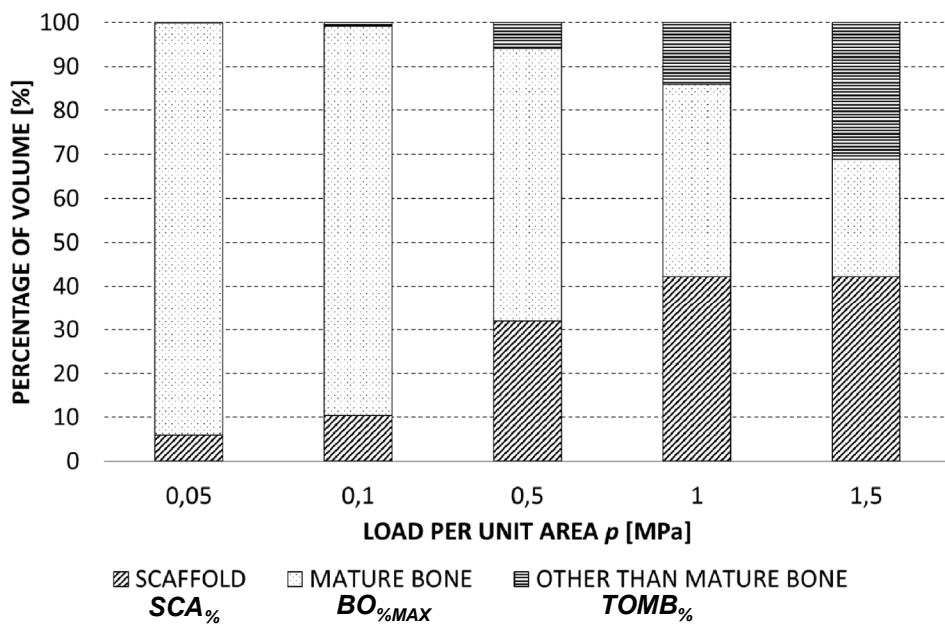
1 Tissues Other than Mature Bone (TOMB) is defined as $TOMB\% = (V_{TOT} - V_{SCAFF} - V_{BONE}) / V_{TOT} \times 100$.
2 Increasing amounts of TOMB are predicted for increasing levels of the load (Fig. 8). However, for
3 all the hypothesized values of pressure, $TOMB\%$ computed for the square cross-section (Fig. 8(b)) is
4 larger than the circular cross-section counterpart (Fig. 8(a)). The percentage of volume occupied by
5 scaffold $SCA\%$ in the case of the circular (Fig. 8(a)) cross-section, instead, appears to be larger than
6 that predicted for the square cross-section (Fig. 8(b)) especially in the case of high load levels. In a
7 not optimized configuration (for example, in Fig. 8(c), the optimized ($D = 58 \mu\text{m}$, Fig. 6(b)) and not
8 optimized ($D = 40 \mu\text{m}$) configuration of a scaffold with circular section, $E = 1500 \text{ MPa}$, $p = 0.1 \text{ MPa}$
9 is shown), larger amounts of immature bone, cartilage and fibrous tissues are predicted to form (Fig.
10 8(c)) compared to an optimized configuration.

11 3.2. Effect of the length L of the beam elements included in the unit cell

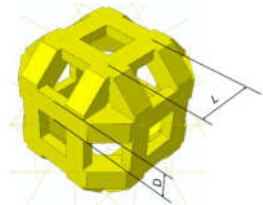
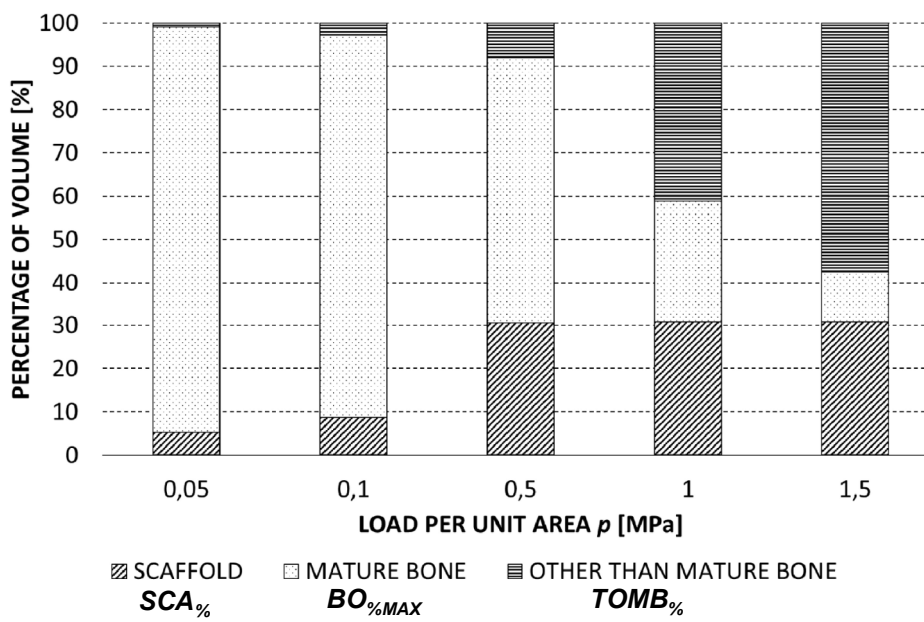
12 Increasing values of L lead to increasing values of D (Figs. 9(a), 9(c), 9(d) and 9(f)), which is
13 consistent with the physics of the problem. In fact, as L increases, the global scaffold stiffness
14 decreases. Therefore, in order to re-establish the original scaffold stiffness, the algorithm increases
15 the dimension D . However, changing the dimension L does not lead to significant changes of $BO\%_{MAX}$.
16 In fact, the curves representing $BO\%_{MAX}$ in function of the load p are practically overlapped (Figs.
17 9(b) and 9(e)), which indicates that $BO\%_{MAX}$ is rather insensitive to the specific value fixed for L . Only
18 the results referring to the case $E = 1000 \text{ MPa}$ are shown in the Figure 9. Diagrams obtained for the
19 other hypothesized scaffold Young's moduli are similar to those shown in Figure 8 and therefore, for
20 the sake of brevity, omitted. Interestingly, for variable values of L , $p_{saturation}$ remains constant (Figs.
21 9(a) and 9(d)), which means that the saturation load does not depend on the dimension L .

22 3.3. Comparison with other scaffold unit cells: performance maps

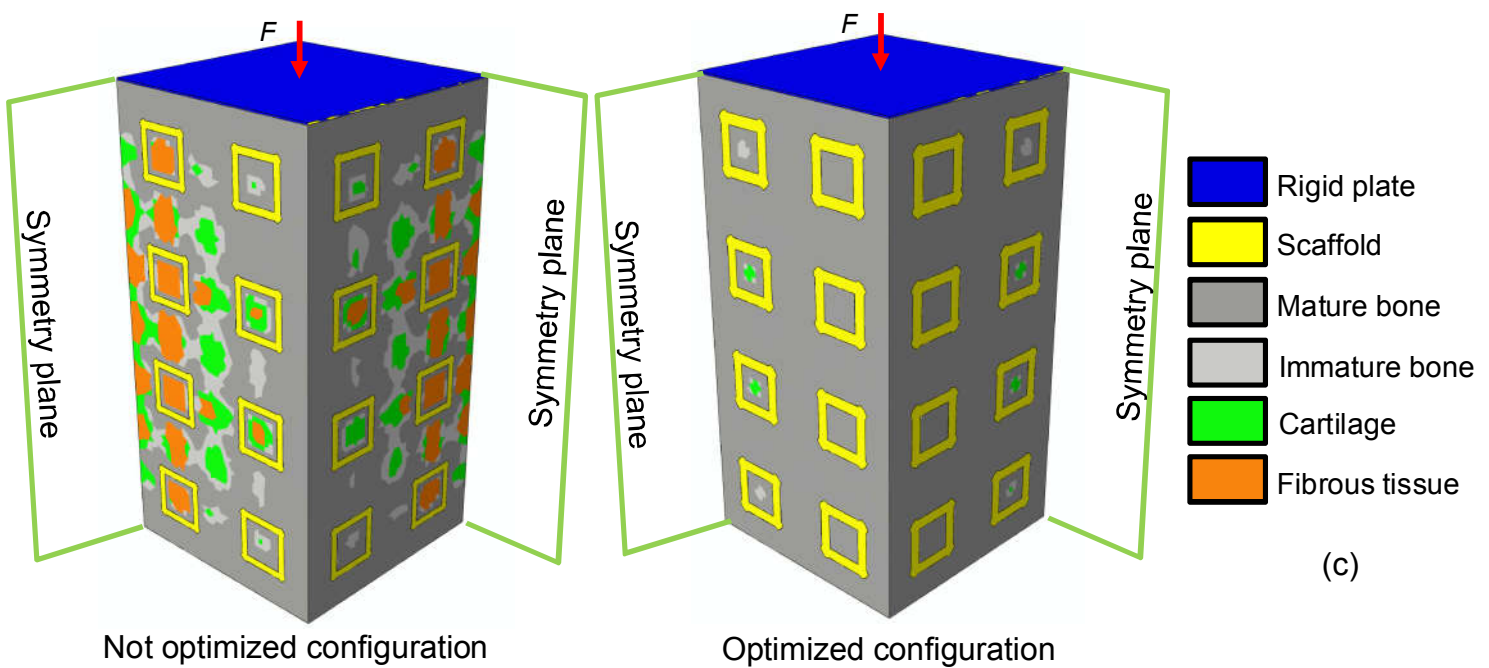
23 In order to determine, for different load levels, the best unit cell that allows maximizing the
24 bone formation, the amounts of bone predicted for different geometries were compared. Depending



(a)



(b)



(c)

Fig. 8. Percentage of volume occupied by mature bone, scaffold and tissues other than mature bone predicted in both, circular (a) and square (b) section, $E = 1000$ MPa. The percentage of volume occupied by mature bone corresponds to $BO_{\%MAX}$. Patterns of the tissues differentiating within the scaffold pores (circular section, $E = 1500$ MPa, $p = 0.1$ MPa) in a not optimized ($D = 40 \mu\text{m}$) and optimized ($D = 58 \mu\text{m}$) scaffold configuration.

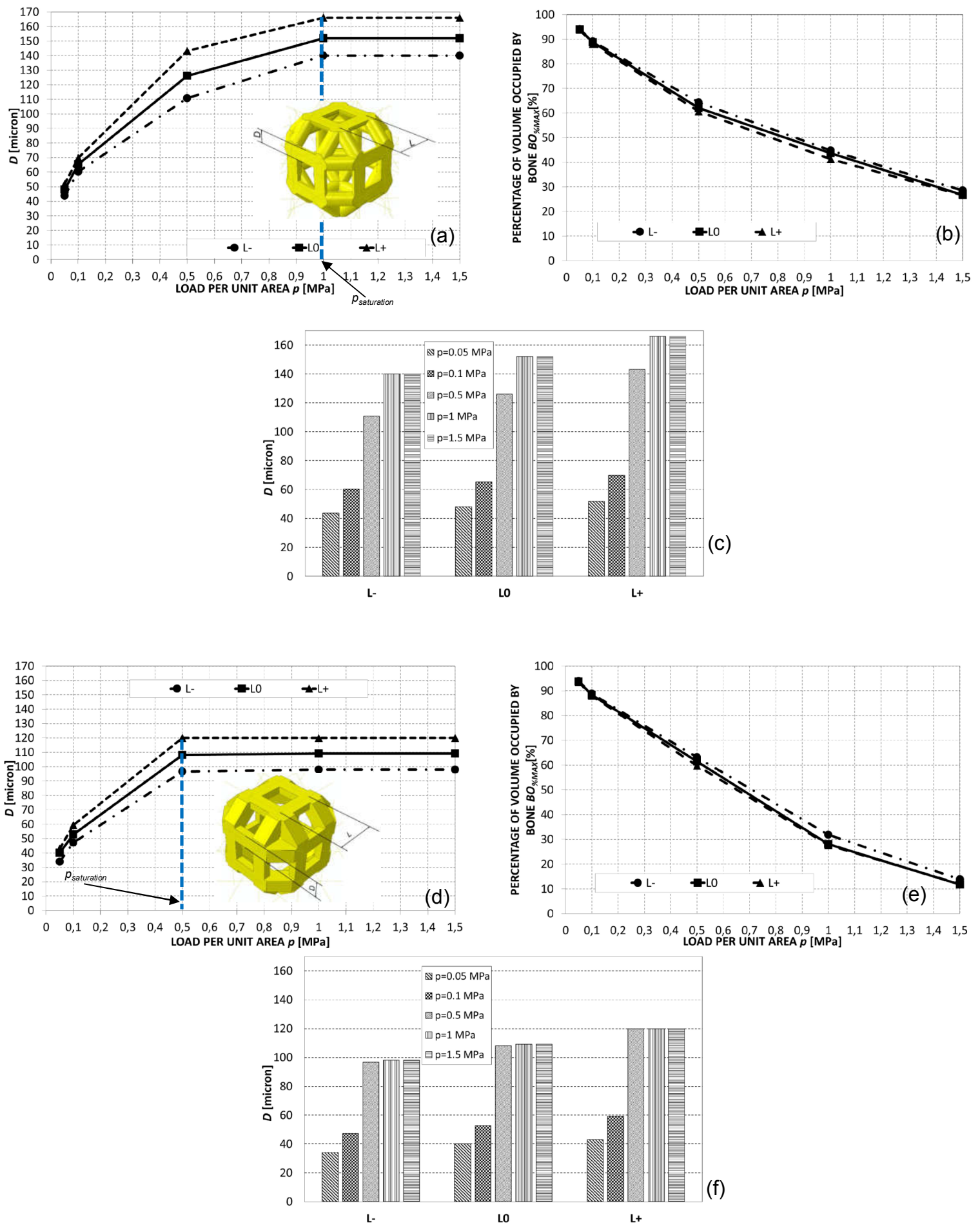


Fig. 9. Values of D (a, d) and $BO_{\%MAX}$ (b, e) predicted by the algorithm for different values of L in the case of circular (a, b) and square (d, e) cross-section. Values of D expressed in function of the dimension L for the circular (c) and square (f) cross-section. Increasing values of L lead to increasing values of D , which is consistent with the physics of the problem. In fact, as L increases, the global scaffold stiffness decreases. Therefore, in order to re-establish the original scaffold stiffness, the algorithm increases the dimension D . For the sake of brevity, only the results referring to $E = 1000$ MPa are shown in the figure.

1 on the compressive load acting on the scaffold, we evaluated if and when a given unit cell is preferable
2 to others. In other words, we traced performance maps that allow to determine, for different values
3 of the compressive load p , the best scaffold type/geometry (Fig. 10).

4 $BO_{\%MAX}$ values obtained for the circular cross-section were compared with those related to the
5 square one (Fig. 10(a)). Different regions highlighted with different colours were identified. For low
6 load values (< 0.5 MPa, i.e. region highlighted in yellow) using a circular cross-section is equivalent
7 to using a square cross-section. For load levels higher than 0.5 MPa, instead (i.e. region highlighted
8 in blue), the circular cross-section performs better than the square one, as for the circular cross-section
9 larger amounts of bone are predicted to form. However, for all the hypothesized levels of load, the
10 volume occupied by the scaffold $SCA_{\%}$ in the case of circular cross-section appears larger than the
11 counterpart predicted for the square one (Fig. 10(b)). $BO_{\%MAX}$ values computed in this study were
12 also compared with those obtained for simpler unit cells (Figs. 10(c) and 10(e)), namely, a hexahedron
13 (or, more precisely, a rectangular cuboid) unit cell obtained by realizing in a cubic volume: (i) Elliptic
14 (or Circular) Pores (EoCP) (Fig. 10(c)) or (ii) Rectangular (or Square) Pores (RoSP) (Fig. 10(e)). In
15 detail, rhombicuboctahedron unit cell/ circular cross-section was compared with EoCP unit cell, while
16 rhombicuboctahedron unit cell/square cross-section with RoSP unit cell. For all the hypothesized load
17 values, RoSP unit cell performs better than the rhombicuboctahedron unit cell/square cross-section
18 (Fig. 10(e), see region highlighted in red). In particular, the hexahedron unit cell with rectangular
19 cross-section presents the smallest values of $SCA_{\%}$ (Fig. 10(f)). Interestingly, for all the hypothesized
20 values of the compressive load p , the scaffold with hexahedron unit cell, rectangular pores, exhibits
21 values of $SCA_{\%}$ smaller than those computed for hexahedron unit cell, square pores. This can be
22 justified with the argument that in the case of rectangular pores, two design variables have to be
23 optimized (i.e. the height and the width of the rectangle) and then greater $BO_{\%MAX}$ values, - or,
24 equivalently, smaller values of $SCA_{\%}$ - can be obtained than the case where just one design variable
25 (i.e. the length of the square side) can be optimized (Fig. 10(f)). For low load levels (≈ 0.2 MPa,
26 region highlighted in green) the rhombicuboctahedron unit cell/circular cross-section performs better

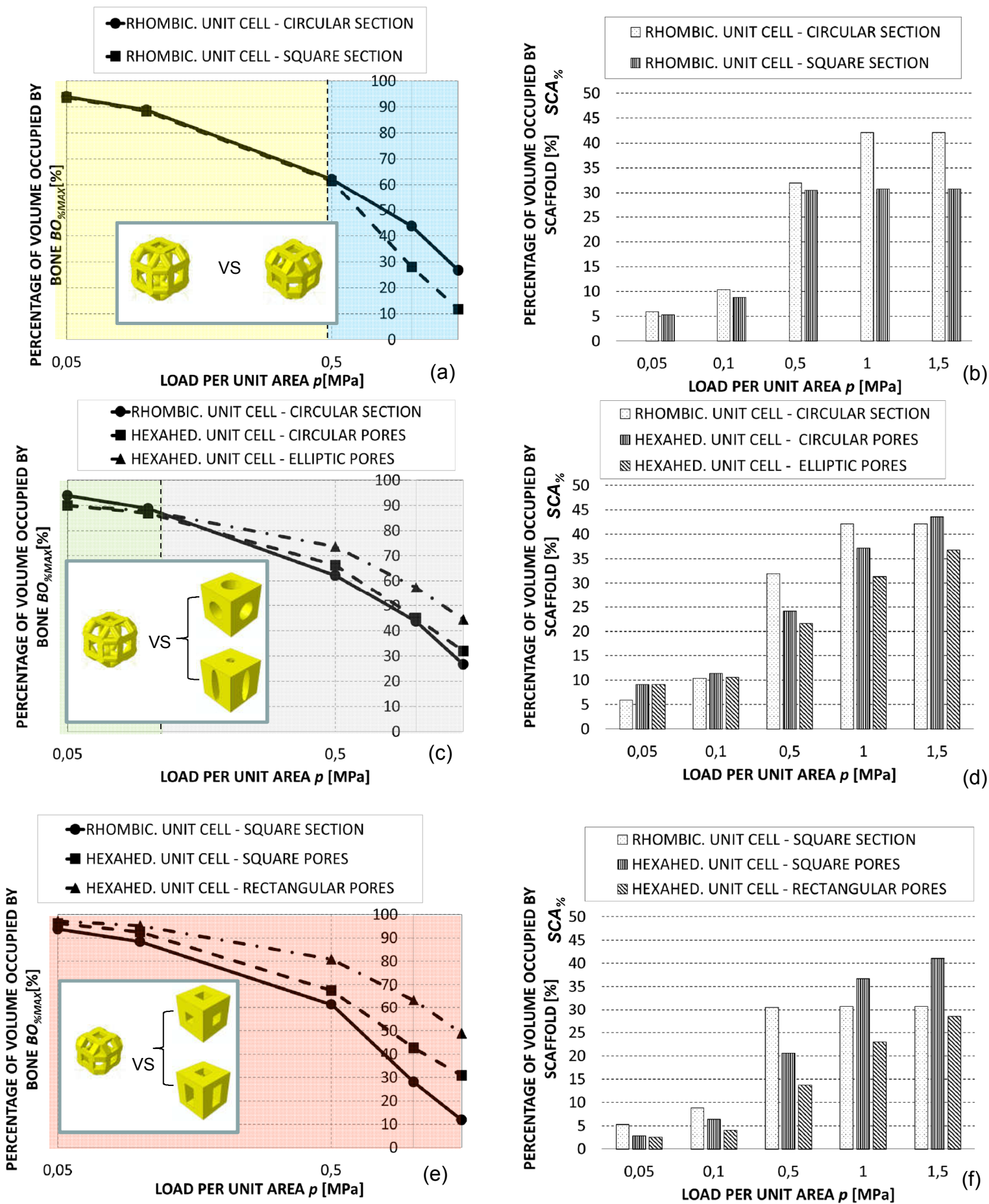


Fig. 10. Performance maps. Comparison between the percentage of bone predicted (a, c, e) and the percentage of volume occupied by scaffold (b, d, f) for different scaffold unit cells: (a-b) rhombicuboctahedron unit cell, circular cross-section vs. rhombicuboctahedron unit cell, square cross-section; (c-d) rhombicuboctahedron unit cell, circular cross-section vs. hexahedron unit cell with circular and elliptic pores (EoCP unit cell, data taken from Boccaccio *et al.* [30]); (e-f) rhombicuboctahedron unit cell, square cross-section vs. hexahedron unit cell with square and rectangular pores (RoSP unit cell, data taken from Boccaccio *et al.* [30]). In order to better visualize the results, a logarithmic scale was utilized on the axis of the load. Different regions highlighted with different colours were identified where a given unit cell geometry is preferable to others.

1 than the EoCP one; for higher loads, instead, the contrary occurs (Fig. 10(c), region highlighted in
2 grey). Analogously, for low values of load, the volume $SCA\%$ predicted for the rhombicuboctahedron
3 unit cell/circular cross-section, appears to be smaller than that computed for EoCP. The contrary
4 occurs for higher values of load (Fig. 10(d)) As observed above in the case of the RoSP unit cell, the
5 volume $SCA\%$ predicted for hexahedron unit cell, elliptic pores is always smaller than that computed
6 for the hexahedron unit cell, circular pores (Fig. 10(d)). Again, this can be justified with the fact that
7 for the elliptic pores two design variables (i.e. the major and the minor axes of the ellipse) has to be
8 optimized while for the circular ones, just one (i.e. the radius of the circumference).

9 The proposed approach presents some limitations. First, a simplified model of the scaffold
10 geometry was utilized. In general, scaffolds utilized in the orthopaedic surgery are much more
11 complex structures with a large number of pores. Modelling such structures would require a
12 computational cost tremendously high thus making its implementation into the clinical context *de*
13 *facto* not affordable. However, predictions carried out with the proposed approach on small portions
14 of the scaffold actually implanted can certainly be performed and give a reasonably good idea about
15 the best scaffold geometry. Second, predictions of the optimal scaffold geometry were carried out
16 based on the values of the biophysical stimulus S registered within the granulation tissue during the
17 early stages of the bone development process. Ideally, the best scaffold geometry should be
18 determined considering the values of S during the entire time window that goes from the scaffold
19 implantation until the completion of the healing process. However, introducing the variable time in
20 the optimization process would make the algorithm much, much more expensive in terms of the
21 computational time and this, again, would make its implementation into the clinical context very
22 critical. Strictly related to this, is the (third) limitation that in the study scaffold dissolution was not
23 modelled. Ideally, for each scaffold geometry proposed by the algorithm as a possible candidate
24 solution, the algorithm itself should simulate the dissolution process in time and based on the scaffold
25 geometry after this time, the algorithm should re-propose new candidate solutions. On the basis of
26 the experience of previous studies [41,50] where we simulated the healing process (in time) of bone

1 fractures, we can estimate that including and simulating the dissolution process would require a
2 computational cost at least two orders of magnitude higher than that spent in the present study. Fourth,
3 angiogenesis, which is another factor that can partially regulate the bone regeneration in scaffolds
4 [51] was not included in the model. Increases in computational power will ultimately allow more
5 realistic models to be developed and the variable time to be taken into account. Fifth, simplified
6 boundary and loading conditions were hypothesized. For instance, a rigid plate was utilized to load
7 the scaffold but, in reality, the scaffold will be presumably loaded by bone or other “biological
8 materials” which possess specific poroelastic properties that were not taken into account in the study.
9 Furthermore, the transport of nutrients and oxygen to the cells occupying the scaffold pores was not
10 simulated [52]. Sixth, new fabrication techniques are recently developing that allow the production
11 of scaffolds within which different types of cells can attach, proliferate and differentiate [53,54].
12 Analyses on these new types of scaffolds should be carried out in the future. Seventh, coherently with
13 the mechano-regulation diagram defined as in Lacroix and Prendergast [49], the predicted values of
14 octahedral shear strain were, for the regions ‘occupied’ by bone, within the interval $[0.04 \div 3.75]$ %.
15 These values are consistent with those measured in osteocytes and osteoblasts in femur samples from
16 rats [21]. Interstitial fluid flow values smaller than $3 \mu\text{m/s}$ were predicted for the regions ‘occupied’
17 by bone while higher values of this velocity were predicted for the regions occupied by cartilage ($<$
18 $9 \mu\text{m/s}$) or fibrous tissue ($> 9 \mu\text{m/s}$). These values are a little bit smaller than the fluid velocity
19 measured within the lacunar/canalicular system of a mechanically loaded bone [18] as well as the
20 average interstitial fluid velocities predicted in osteocyte models subjected to loading levels
21 representative of vigorous physiological activity [20]. However, the boundary and loading conditions
22 utilized in these studies [18,20] are very different with respect to those hypothesized in this article.
23 Eighth, for the values of load p considered in this study, the parameter S always had to be reduced
24 (Fig. 6(c) and 7(c)). For instance, for decreasing levels of the Young’s modulus the global scaffold
25 stiffness decreases too, which leads to high values of S . The algorithm, then, tends to counterbalance
26 this, increasing the cross-section dimension D thus reducing the biophysical stimulus S . However, we

1 expect that for lower levels of p , the contrary occurs, i.e. S has to be increased to avoid that in some
2 regions within the scaffold pores, the bone resorption process takes place. This behavior was
3 observed, for example, for the hexahedron unit cell [39] where, for loads $p \leq 0.01$ MPa, in large
4 portions of the granulation tissue, very small values of S were predicted. This led the optimizer to
5 increase as much as possible the dimensions of the pores thus making the scaffold more compliant
6 and, consequently, increasing the average value of the biophysical stimulus S . In the present study,
7 values of pressure so small were not considered for the following twofold reasons. (i) Considering
8 loads $p \leq 0.01$ MPa, the optimizer predicts rhombicuboctahedron unit cells with very thin beam
9 elements, which require a very very fine mesh. Preliminary investigations showed that the meshing
10 these very thin structures requires at least $10 \div 15 \times 10^6$ elements with computational times
11 tremendously longer than those spent in this study. (Note. In the case of the hexahedron unit cell, the
12 meshing process of scaffolds with pores with increasing dimensions did not require to change the
13 element dimensions as, instead, occurs in the case of the rhombicuboctahedron unit cell). (ii) Values
14 of load $p \leq 0.01$ MPa have scarce relevance from the practical point of view as they are more than
15 three orders of magnitude smaller than the ultimate strength in compression of the spongy bone [42]
16 and hence significantly smaller than any actual physiological load.

17 In spite of these limitations, we observe that the predictions of the proposed model are
18 consistent (Fig. 10) with those of previous studies [39] that successfully predicted the best geometry
19 of scaffolds with hexahedron unit cells. Predictions of the proposed model are also consistent with
20 the solutions of the analytical model proposed by Hedayati *et al.* [31]. In detail, in order to compare
21 the results of the proposed model with those of the Hedayati *et al.* one, the following steps were
22 followed. The only scaffold model (granulation tissue excluded) was first generated (with the
23 following values of $D = 60, 80, 100$ and $120 \mu\text{m}$ for the circular cross-section and $D = 60, 80$ and 100
24 μm for the square cross-section) and then subjected to a compression load $p = 0.1$ MPa (Fig. 11). The
25 resulting displacement fields u_2 were predicted in both cases, circular and square cross-section (Fig.
26 11(b)). The usual definition of elastic modulus was then employed to determine the apparent Young's

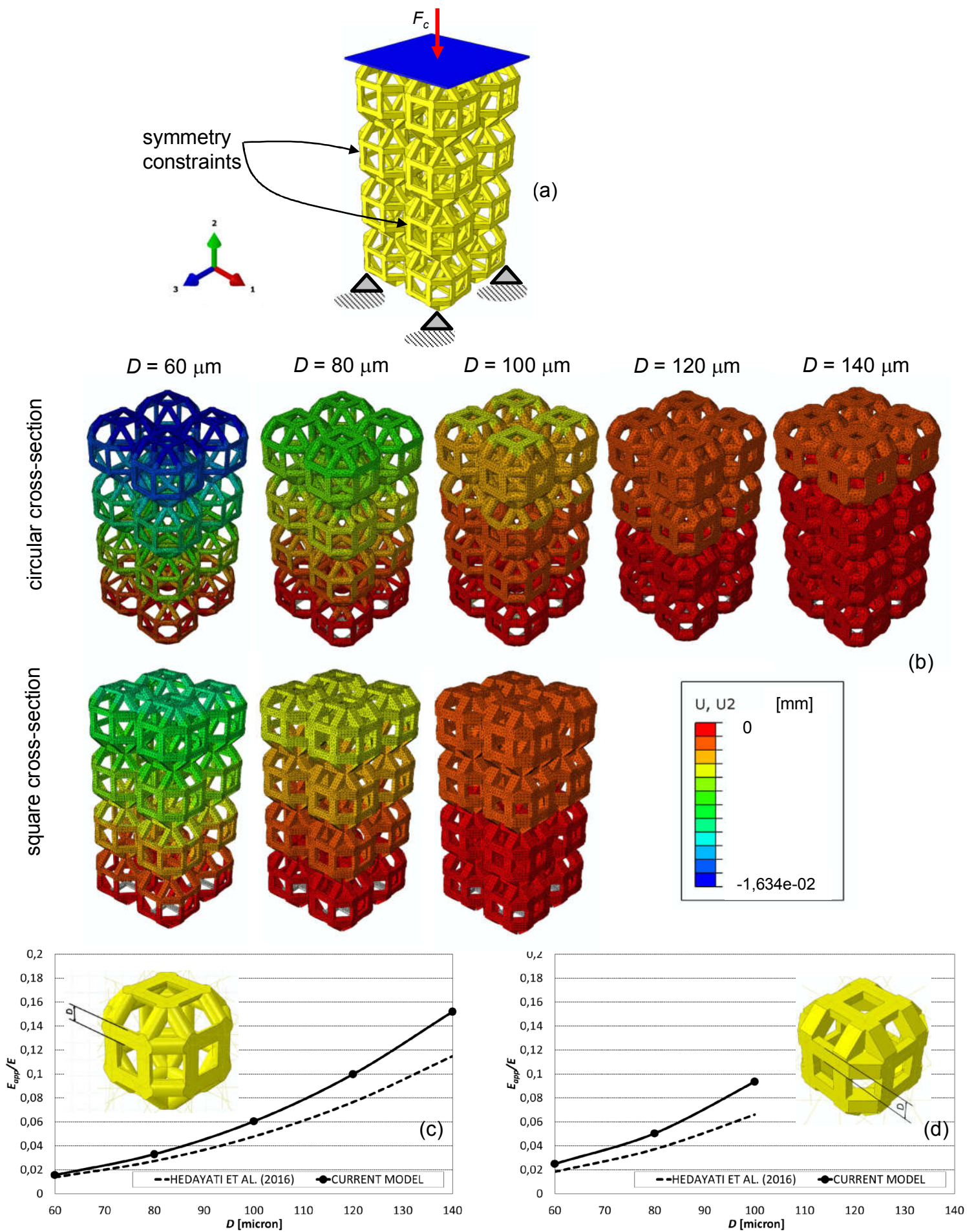


Fig. 11. Corroboration/validation of the proposed model. The scaffold model was subjected to a compression load $p = 0.1$ MPa (a). The resulting displacement fields u_2 were predicted for different values of D in both, circular and square cross-section (b). The usual definition of elastic modulus was employed to determine the apparent Young's modulus E_{app} for the scaffold structure. Based on the FE predictions, the ratio E_{app}/E was computed for different levels of D and compared with that obtained via the analytical solution by Hedayati *et al.* [22]. Comparison was carried out for both, circular (c) and square (d) cross-section.

1 modulus E_{app} for the scaffold structure. Specifically, if u_2 is the displacement along the direction 2
2 (Fig. 11(a)) of the rigid plate after the application of the load (per unit area) p , the apparent Young's
3 modulus is given by: $E_{app} = p \cdot h / u_2$, being h the height of the scaffold model (Fig. 2). Based on the FE
4 predictions, the ratio E_{app}/E was computed for different levels of D and compared with that obtained
5 via the analytical solution by Hedayati *et al.* [31] that is briefly summarized in the Appendix.
6 Interestingly, for small values of D , i.e. for the condition of slender beam, predictions of our model
7 are close to those obtained via the analytical model. However, as we move towards the condition of
8 large values of D , i.e. the condition of deep beam, the analytical solution starts to deviate, which is
9 consistent with our expectations in consideration of the fact that the Euler-Bernoulli theory (on which
10 the analytical solution is based), does not account for the effect of slenderness/depth of beams. It is
11 worthy to note that the computed values of the ratio E_{app}/E are consistent with those of the trabecular
12 bone tissue. Assuming an apparent Young's modulus for the spongy bone of $E_{app_trab_bone} = 580$ MPa
13 (i.e. the average value of the experimental values measured by Odgaard and Linde [55]) and an elastic
14 modulus of individual trabeculae of $E_{trabeculae} = 12600$ MPa (i.e. the average of the experimental
15 values measured by Rho *et al.* [56]) the ratio $E_{app_trab_bone} / E_{trabeculae} = 0.046$ can be computed, which
16 falls within the range of the values of E_{app}/E computed in this study (Figs. 11(c) and (d)).

17 The patterns of bony tissue predicted with the proposed model are also consistent with those
18 observed in vivo in three dimensional matrix channels with different shapes [4]. In Figure 12 the
19 experimental patterns of new tissue formed in square and triangular channels are compared to those
20 predicted in square and triangular elements of the single rhombicuboctahedron unit cell. Specifically,
21 grey elements shown in the figure are those for which the condition $n_{resorb} < S < n_{mature}$ is satisfied
22 (i.e., formation of mature bone). Bone formation starts from the matrix wall and proceeds towards
23 the centre while the sharp edges of the matrix are 'rounded' by the new formed tissue. This same
24 behaviour was predicted by the proposed model (Figs. 12(a) and 12(b)).

25 The proposed algorithm well suits to the recent research lines more and more oriented towards
26 customized medical solutions. Based on the antropometric data of the specific patient and on the

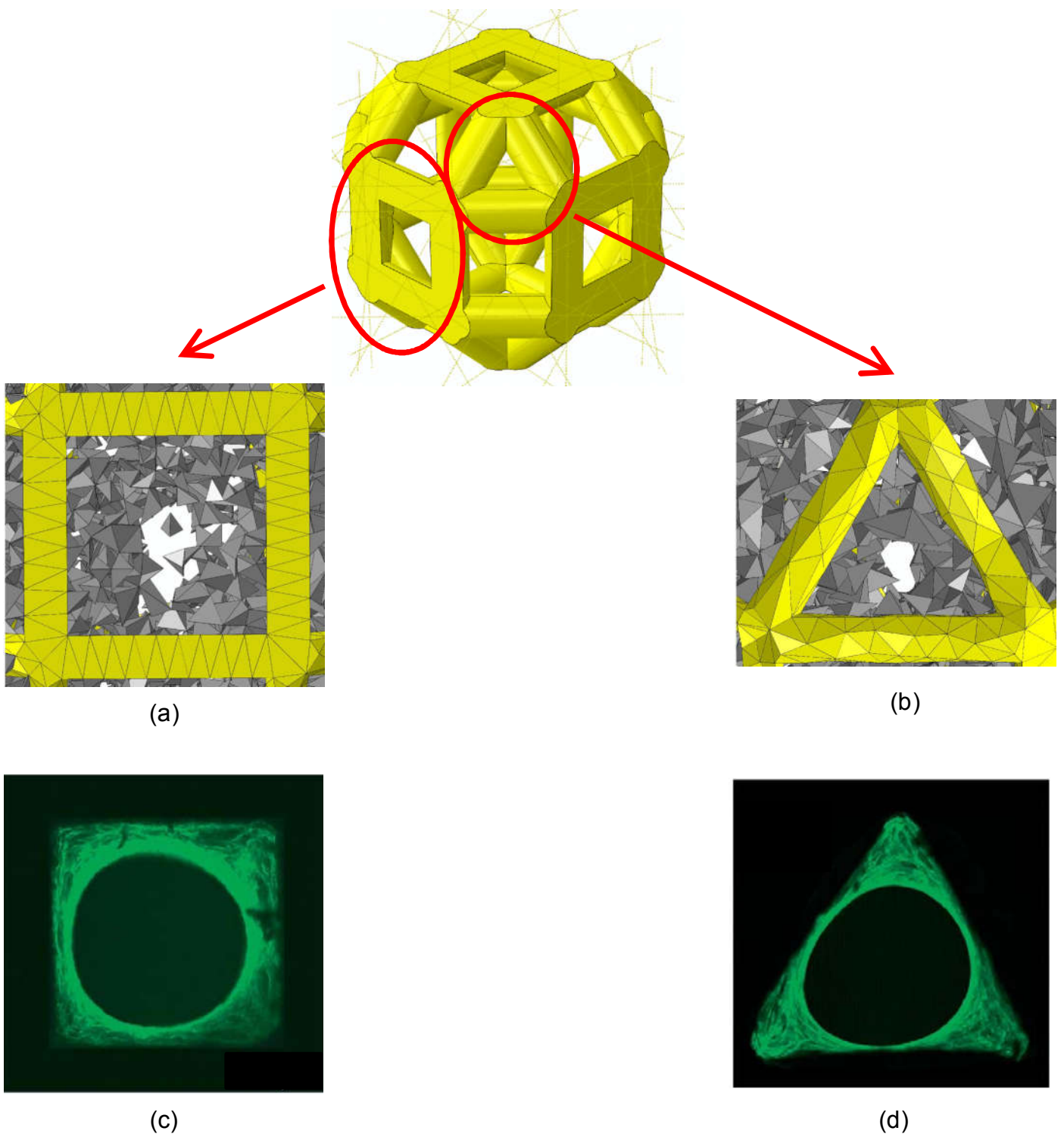


Fig. 12. Corroboration/validation of the proposed model. Patterns of bony tissue predicted to form within square (a) and triangular (b) elements of the rhombicuboctahedron unit cell compared with patterns of new tissue formed in three-dimensional matrix channels with square (c) and triangular (d) shape (Figures (c) and (d) have been taken from Rumpler *et al.* [4]).

1 morphology of the specific anatomic region - where scaffold must be implanted -, the possible
2 boundary and loading conditions acting on the scaffold itself can be hypothesized. **Therefore, utilizing**
3 **the proposed algorithm, one could determine the optimal geometry design that encourages bone**
4 **growth thus maximizing the bone formation.** The performance maps (Fig. 10) traced with the
5 proposed algorithm, can guide the orthopaedic/surgeon in the choice of the best scaffold to be
6 implanted into a patient-specific anatomic region.

7 **4. Conclusions**

8 A mechanobiology-based optimization algorithm was developed to determine the best geometry of
9 scaffolds for bone tissue engineering made from rhombicuboctahedron unit cells. In spite of the huge
10 number of articles on regular porous biomaterials made from different unit cells, mechanobiology of
11 tissue differentiation in such scaffolds remains unclear. We proposed the first study ever reported in
12 the literature where the best geometry of scaffolds with rhombicuboctahedron unit cell was
13 determined via a mechanobiology-based optimization algorithm.

14 For different cross-section shapes and for different values of the: (i) scaffold Young's modulus,
15 (ii) length of the beam elements included in the unit cell and (iii) compressive load acting on the
16 scaffold upper surface, the optimal cross-section dimension D was determined.

17 The algorithm predicts that the circular cross-section performs better than the square one. The
18 scaffold performances appear rather insensitive to the length of the beam elements included in the
19 unit cell.

20 We compared the performances of the proposed scaffold type with those of other scaffolds
21 based on simple hexahedron unit cells. **In detail, the amounts of bone and the solid volume fraction**
22 **were compared.** We also identified different load levels where the use of a given unit cell is preferable
23 to others and vice-versa. We found that for high compressive loads, scaffolds with hexahedron unit
24 cell perform better than those utilizing the rhombicuboctahedron one. For low load values, instead,

1 rhombicuboctahedron unit cell exhibits performances comparable and even better than those of
 2 hexahedron unit cell.

3 The bony patterns predicted with the proposed model appear reasonably consistent with those
 4 observed in *in vitro* studies.

5 In a context that becomes more and more oriented towards customized solutions the proposed
 6 algorithm appears a promising tool. Based on the anthropometric data of the specific patient as well
 7 as on the specific anatomy of the region where the scaffold must be implanted, one can hypothesize
 8 the possible values of the load acting on the scaffold structure and, implementing the proposed model,
 9 ‘identify’ the best scaffold that is better suited to the specific scope. The performance maps traced
 10 with the proposed algorithm, can guide the orthopaedic/surgeon in the choice of the best scaffold to
 11 be implanted into a patient-specific anatomic region.

12

13 Appendix

14

15

16 Based on the Euler-Bernoulli beam theory, Hedayati *et al.* [31] related for both, the circular and the
 17 square cross-section, the apparent Young’s modulus E_{app} with the scaffold Young’s modulus E
 18 through the quantities D , L_0 and the apparent Poisson’s ratio ν_{app} . The analytical solution for circular
 19 cross-section with radius $r = D/2$ is given by the following system of equations:

20

$$\left\{ \begin{array}{l} \frac{E_{app}}{E} = \frac{4\pi \left(\frac{r}{L_0}\right)^2}{3(1+\sqrt{2})} \left[\frac{4 + 108\left(\frac{r}{L_0}\right)^2 + 207\left(\frac{r}{L_0}\right)^4 + 81\left(\frac{r}{L_0}\right)^6 + \frac{1}{2(1+\nu_{app})} \left(\frac{2}{3} + 19\left(\frac{r}{L_0}\right)^2 + 45\left(\frac{r}{L_0}\right)^4 + 18\left(\frac{r}{L_0}\right)^6\right)}{8 + 70\left(\frac{r}{L_0}\right)^2 + 105\left(\frac{r}{L_0}\right)^4 + 27\left(\frac{r}{L_0}\right)^6 + \frac{1}{2(1+\nu_{app})} \left(\frac{4}{3} + 13\left(\frac{r}{L_0}\right)^2 + 23\left(\frac{r}{L_0}\right)^4 + 6\left(\frac{r}{L_0}\right)^6\right)} \right] \\ r = \frac{D}{2} \\ \nu_{app} = \frac{1}{3} \frac{8 - 12\left(\frac{r}{L_0}\right)^2 - 36\left(\frac{r}{L_0}\right)^4 + \frac{1}{2(1+\nu_{app})} \left(\frac{4}{3} - \left(\frac{r}{L_0}\right)^2 - \left(\frac{r}{L_0}\right)^4\right)}{8 + 70\left(\frac{r}{L_0}\right)^2 + 105\left(\frac{r}{L_0}\right)^4 + 27\left(\frac{r}{L_0}\right)^6 + \frac{1}{2(1+\nu_{app})} \left(\frac{4}{3} + 13\left(\frac{r}{L_0}\right)^2 + 23\left(\frac{r}{L_0}\right)^4 + 6\left(\frac{r}{L_0}\right)^6\right)} \end{array} \right. \quad Eq. (A.1)$$

22

23

24 Analogously, the analytical solution for square cross-section with side long D is given by:

25

$$\left\{ \begin{array}{l}
1 \quad \frac{E_{app}}{E} = \frac{4 \left(\frac{D}{L_0}\right)^2}{(1 + \sqrt{2})} \left[\frac{12 + 108 \left(\frac{D}{L_0}\right)^2 + 69 \left(\frac{D}{L_0}\right)^4 + 9 \left(\frac{D}{L_0}\right)^6 + \frac{1}{2(1 + \nu_{app})} \left(2 + 19 \left(\frac{D}{L_0}\right)^2 + 15 \left(\frac{D}{L_0}\right)^4 + 2 \left(\frac{D}{L_0}\right)^6\right)}{72 + 210 \left(\frac{D}{L_0}\right)^2 + 105 \left(\frac{D}{L_0}\right)^4 + 9 \left(\frac{D}{L_0}\right)^6 + \frac{1}{2(1 + \nu_{app})} \left(12 + 39 \left(\frac{D}{L_0}\right)^2 + 23 \left(\frac{D}{L_0}\right)^4 + 2 \left(\frac{D}{L_0}\right)^6\right)} \right] \\
2 \quad \nu_{app} = \frac{24 - 12 \left(\frac{D}{L_0}\right)^2 - 12 \left(\frac{D}{L_0}\right)^4 + \frac{1}{2(1 + \nu_{app})} \left(4 - \left(\frac{D}{L_0}\right)^2 - 3 \left(\frac{D}{L_0}\right)^4\right)}{72 + 210 \left(\frac{D}{L_0}\right)^2 + 105 \left(\frac{D}{L_0}\right)^4 + 9 \left(\frac{D}{L_0}\right)^6 + \frac{1}{2(1 + \nu_{app})} \left(12 + 39 \left(\frac{D}{L_0}\right)^2 + 23 \left(\frac{D}{L_0}\right)^4 + 2 \left(\frac{D}{L_0}\right)^6\right)} \\
3 \\
4 \\
5
\end{array} \right. \quad Eq. (A.2)$$

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- 5

1 **Figure legends**

2 **Fig. 1.** The generation of the rhombicuboctahedron unit cell was performed in seven subsequent steps
3 (a-g). In the first step (a), an octagonal trajectory t_{oct} was traced, a circular or square cross-section
4 was created and then, through the tool ‘sweep’ available in ABAQUS, a solid with the fixed cross-
5 section was protruded along t_{oct} . All the sides of the octagonal trajectory t_{oct} were hypothesized to be
6 equal and long L . The same procedure described in the first step (a) was successively repeated in the
7 steps 2 to 5 ((b) to (f)) by adding a solid volume (protruded along t_{oct}) in each step. In the step 7 (g)
8 the solid so obtained was cut along planes π_{Mi} ($i=1, 2, \dots, 6$) delimiting the cubic volume ($Q \times Q \times Q$,
9 $Q = L \times (1 + 2 \cos(\pi/4))$) containing the unit cell. Rhombicuboctahedron unit cells with circular (h)
10 and square (m) cross-section investigated in the study with indicated the dimensions L and D
11 parameterized in the study. Three different values of L were hypothesized: $L = L_0 = 263,85 \mu\text{m}$, $L =$
12 $L_- = L_0 \times 0,9$ and $L = L_+ = L_0 \times 1,1$. The entire scaffold geometry was obtained by successively
13 mirroring the unit cell with respect to π_{Mi} planes.

14
15 **Fig. 2.** Boundary and loading conditions acting on the scaffold model. CAD model of the scaffold
16 (a), granulation tissue (b) and entire (including 64 unit cells) system (scaffold + granulation tissue)
17 (c). Exploiting the symmetry of the problem, a simplified model reproducing only 1/4 of the entire
18 geometry was considered (d). (e) Detailed views of the simplified model with indicated the boundary
19 and loading conditions acting on it. As the surrounding tissue would not totally prevent the fluid from
20 escaping, the pore pressure p_{gr_tis} on the outer surfaces of the granulation tissue was set equal to zero
21 to simulate the free exudation of fluid. On the symmetry planes instead, being null the fluid flow, the
22 pore pressure must be $p_{sym} = \infty$.

23
24 **Fig. 3.** CAD model of the scaffold (a, f) and of the granulation tissue (b, g) in the case of circular (a,
25 b) and square (f, g) cross-section. Finite element mesh of the scaffold (c, h), the granulation tissue (d,

1 m) and of the entire system (e, n) in the case of circular (c, d, e) and square (h, m, n) cross-section.
2 The entire model (scaffold + granulation tissue) included about 5×10^6 elements with about 1×10^6
3 nodes.

4
5 **Fig. 4.** Schematic of the algorithm written in Matlab environment to determine the optimal dimension
6 D for different values of load, Young's modulus and L and for different cross-section shapes.

7
8 **Fig. 5.** (a) Upper and lower bounds set for the dimension D for different cross-section shapes and
9 values of L . Geometric configuration of the model for $D = D_{min}$ (b) and $D = D_{max}$ in the case of circular
10 (c) and square (d) cross-section. Values of $D > D_{max}$ lead to close the aperture between the inclined
11 sides (see detailed images (c) and (d)) thus changing the originally hypothesized connectivity
12 scaffold.

13
14 **Fig. 6.** Optimization of the rhombicuboctahedron unit cell, circular cross-section. (a) Optimal scaffold
15 geometries predicted by the algorithm, in the case of circular cross-section. Values of the optimal
16 diameter D (b) and of $BO\%MAX$ (c) predicted by the algorithm for different levels of load and scaffold
17 Young's modulus. For the sake of clarity, the only saturation load $p_{saturation}$ related to $E = 500$ MPa is
18 shown in the figure.

19
20 **Fig. 7.** Optimization of the rhombicuboctahedron unit cell, square cross-section. (a) Optimal scaffold
21 geometries predicted by the algorithm, in the case of square cross-section. Values of the optimal
22 diameter D (b) and of $BO\%MAX$ (c) predicted by the algorithm for different levels of load and scaffold
23 Young's modulus. For the sake of clarity, the only saturation load $p_{saturation}$ related to $E = 500$ MPa is
24 shown in the figure.

1 **Fig. 8.** Percentage of volume occupied by mature bone, scaffold and tissues other than mature bone
2 predicted in both, circular (a) and square (b) section, $E = 1000$ MPa. The percentage of volume
3 occupied by mature bone corresponds to $BO_{\%MAX}$. Patterns of the tissues differentiating within the
4 scaffold pores (circular section, $E = 1500$ MPa, $p = 0.1$ MPa) in a not optimized ($D = 40$ μm) and
5 optimized ($D = 58$ μm) scaffold configuration.

6
7 **Fig. 9.** Values of D (a, d) and $BO_{\%MAX}$ (b, e) predicted by the algorithm for different values of L in
8 the case of circular (a, b) and square (d, e) cross-section. Values of D expressed in function of the
9 dimension L for the circular (c) and square (f) cross-section. Increasing values of L lead to increasing
10 values of D , which is consistent with the physics of the problem. In fact, as L increases, the global
11 scaffold stiffness decreases. Therefore, in order to re-establish the original scaffold stiffness, the
12 algorithm increases the dimension D . For the sake of brevity, only the results referring to $E = 1000$
13 MPa are shown in the figure.

14
15 **Fig. 10.** Performance maps. Comparison between the percentage of bone predicted (a, c, e) and the
16 percentage of volume occupied by scaffold (b, d, f) for different scaffold unit cells: (a-b)
17 rhombicuboctahedron unit cell, circular cross-section vs. rhombicuboctahedron unit cell, square
18 cross-section; (c-d) rhombicuboctahedron unit cell, circular cross-section vs. hexahedron unit cell
19 with circular and elliptic pores (EoCP unit cell, data taken from Boccaccio *et al.* [39]); (e-f)
20 rhombicuboctahedron unit cell, square cross-section vs. hexahedron unit cell with square and
21 rectangular pores (RoSP unit cell, data taken from Boccaccio *et al.* [39]). In order to better visualize
22 the results, a logarithmic scale was utilized on the axis of the load. Different regions highlighted with
23 different colours were identified where a given unit cell geometry is preferable to others.

24
25 **Fig. 11.** Corroboration/validation of the proposed model. The scaffold model was subjected to a
26 compression load $p = 0.1$ MPa (a). The resulting displacement fields u_2 were predicted for different

1 values of D in both, circular and square cross-section (b). The usual definition of elastic modulus was
2 employed to determine the apparent Young's modulus E_{app} for the scaffold structure. Based on the
3 FE predictions, the ratio E_{app}/E was computed for different levels of D and compared with that
4 obtained via the analytical solution by Hedayati *et al.* [31]. Comparison was carried out for both,
5 circular (c) and square (d) cross-section.

6
7 **Fig. 12.** Corroboration/validation of the proposed model. Patterns of bony tissue predicted to form
8 within square (a) and triangular (b) elements of the rhombicuboctahedron unit cell compared with
9 patterns of new tissue formed in three-dimensional matrix channels with square (c) and triangular (d)
10 shape (Figures (c) and (d) have been taken from Rumpler *et al.* [4]).