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Complete List of Authors:	Zadpoor, Amir A; Delft University of Technology (TUDelft), Department of Biomechanical Engineering

Review article

Bone tissue regeneration: the role of scaffold geometry

Amir A. Zadpoor[1*]

[*]*Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology (TU Delft), Mekelweg 2, Delft 2628 CD, The Netherlands*

[1] Corresponding author, email: a.a.zadpoor@tudelft.nl, tel: +31-15-2781021, fax: +31-15-2784717.

ABSTRACT

The geometry of porous scaffolds that are used for bone tissue engineering and/or bone substitution is recently shown to significantly influence the cellular response and the rate of bone tissue regeneration. Most importantly, it has been shown that rate of tissue generation increases with curvature and is much larger on concave surfaces as compared to convex and planar surfaces. In this work, recent discoveries concerning the effects of geometrical features of porous scaffolds such as surface curvature, pore shape, and pore size on the cellular response and bone tissue regeneration process have been reviewed. In addition to reviewing the recent experimental observations, we discuss the mechanisms through which geometry affects the bone tissue regeneration process. Of particular interest are the theoretical models that have been developed to explain the role of geometry in the bone tissue regeneration process. We then follow with a section on the implications of observed phenomena for geometrical design of porous scaffolds including the application of predictive computational models in geometrical design of porous scaffolds. Moreover, some geometrical concepts in the design of porous scaffolds such as minimal surfaces and porous structures with geometrical gradients that have not been explored before are suggested for future studies. We specially focus on the porous scaffolds manufactured using additive manufacturing techniques where the geometry of the porous scaffolds could be precisely controlled. The paper concludes with a general discussion of the current state-of-the-art and recommendations for future research.

Keywords: Regenerative medicine, morphological effects, porous scaffolds, and cell mechanics.

1. INTRODUCTION

In regenerative medicine approaches, porous scaffolds or implants are often used for supporting the regeneration of bone tissue. The role of such porous structures is particularly important in treatment of 'segmental bone defects' [1-4] where the defect size is so large that the natural healing process cannot bridge the gap without the presence of such supporting scaffolds. The Holy Grail in the treatment of large segmental defects is to come up with a biomaterial system that ensures fast and full regeneration of functional bone tissue in the segmental defect. The biomaterial system may include not only the scaffold but also possible drug delivery devices [5] that come with the scaffold and release growth factors, cytokines, or other biological agents that could stimulate and guide the process of bone regeneration.

Stimulating and guiding the process of bone regeneration is not limited to the biological factors. The scaffold itself should be optimally designed not only to 'support' the unhindered progress of bone regeneration process including angiogenesis, but also to 'stimulate' and 'guide' the process. In order to 'support' the bone tissue regeneration process, the porous scaffold should be made of a biocompatible material, be able to provide enough mechanical support, and provide ample interconnected pore space for tissue invasion, cell nutrition, and oxygenation particularly before the completion of angiogenesis.

The second role of the scaffolds, i.e. stimulating and guiding the bone tissue regeneration process, calls for other design requirements. The design of the scaffold should be such that it provides the cues required to stimulate cells to generate *de novo* bone tissue. The scaffold should also guide the process in the right direction so that the *de novo* tissue is regenerated in and/or remodeled to the original anatomical shape of the segmental defect. Several approaches have been proposed for incorporating the appropriate physical cues in the design of scaffolds. For example, a large number of studies have shown that surface (nano-) topography could determine the stem cell fate, facilitate stem cell differentiation towards the

osteogenic lineage, and ultimately result in larger volumes of *de novo* bone formation [6-10]. Another important factor is substrate stiffness that has been shown to significantly influence the cellular response [11-15] and, thus, the process of bone tissue regeneration.

Recently, the role of geometrical design of porous scaffolds in stimulating and guiding the bone regeneration process has received a lot of attention. It has been known already for quite some time that such parameters as pore size and interconnectivity of the porous structure influence the bone regeneration process [16]. However, the role of pore size and interconnectivity were seen as a part of the above-mentioned 'supporting' role of porous scaffolds. For example, it was shown that a minimum pore size of around ≈100 μm is required for successful progression of the bone regeneration process [16]. A porous structure with smaller pore size and/or less interconnected porous structure may not be able to provide enough space for bone ingrowth or be incapable of providing enough space for material transport across the scaffold, before angiogenesis is complete and blood vessels could take over. The influence of the geometrical design of porous scaffolds on their 'supporting' role is therefore not necessarily new. It is, however, the 'stimulating' and 'guiding' role of geometrical design that has recently received recognition and attention. Recent evidence, for example, shows that pore shape [17, 18] and surface curvatures [17, 19-21] could strongly influence the bone tissue regeneration process. Since recent advances in additive manufacturing have enabled precise manufacturing of any porous structure (Figure 1), now is a good time to exploit the potential of geometrical design of scaffolds for enhancing their bone tissue regeneration performance.

This paper presents the recent progress in understanding the role of geometrical design of porous scaffolds not only in 'supporting' the bone tissue regeneration process but particularly in 'stimulating' and 'guiding' the process. The experimental observations regarding the effects of geometry on bone tissue regeneration, the mechanisms through which geometry

affects bone tissue regeneration, and the implications for porous scaffolds design are reviewed.

2. EFFECTS OF GEOMETRY ON TISSUE REGENERATION

There are several geometrical features that are shown to influence the performance of porous scaffolds. In particular, surface curvature, pore shape, and pore size have received the most attention. In this section, we will review the most important observations regarding the effects of the above-mentioned geometrical features on the bone tissue regeneration process.

2.1. Curvature and pore geometry

A number of recent studies have shown that curvature could control and guide the tissue regeneration process. Two types of studies are distinguishable in this regard. The first category of studies includes the ones [22, 23] that study the effects of curvatures at the nano-meter range and single cell level. Those studies are therefore largely looking at the sub-cellular geometrical features that are better categorized under surface topography. As previously mentioned, the effects of surface (nano-) topography on cellular and tissue regeneration have been extensively studied and reviewed elsewhere [24-29]. In this work, we are mainly interested in the second category of studies where the effects of substrate curvature at the larger scale are considered.

The pioneering work on how curvature influences tissue regeneration was performed by Rumpler et al [21]. They showed that curvatures with radii much larger than the cells could interact with the cells and influence their behavior (Figure 2) [21]. This fundamental discovery has important consequences in terms of the optimal design requirements for porous scaffolds and has been confirmed in a series of studies [17, 19, 20, 30, 31]. There is currently only limited evidence available as to what kind of curvature is the best for tissue regeneration. Nevertheless, some observations regarding the effect of curvature have been confirmed in several studies. For example, it has been consistently observed that the rate of tissue

generation is proportional to the curvature of the surface (Figure 2) [17, 19-21]. Moreover, the type of curvature, i.e. concave vs. convex, is also found to be important with some studies showing that the tissue growth process prefers concave surfaces to convex and flat ones (Figure 3c) [19, 20, 32-35]. Furthermore, the density of both actin fiber and myosin lib is found to be higher on concave surfaces, suggesting a locally higher state of cell stress in the neighborhood of concave surfaces (Figure 3d). It has been also observed that variation in surface curvature could cause significant changes in terms of cell attachment rate, cell migration speed, and cell morphology including cell spread area [30, 31]. *In vitro* mineralization is shown to happen inside the cavities created on the surface of calcium phosphate ceramics and not on the planar surfaces [36]. Finally, the mineralization process is found to be controlled by the concavity size created on the surface of calcium phosphate [36]. Most of the above-mentioned studies are performed *in vitro*. There is, nevertheless, some limited evidence from *in vivo* studies that also confirms the role of curvature and curvature sign (concave vs. convex surfaces) in the process of bone tissue regeneration. A histological study that evaluated the number of blood vessels inside concavities and around convexities of an implant in a rabbit model found strong statistical evidence that, in the first few weeks after implantation, blood vessels are concentrated in concavities (Figure 3a-b) [37]. Since angiogenesis is an important part of the bone tissue regeneration process [38], improved progress of angiogenesis could have important consequences in terms of the rate of bone tissue regeneration. In another *in vivo* study in which critical-sized bone defects were treated by a porous scaffold, the arrangement of the fibrous tissue was found to be dependent on the geometrical features of the scaffold [39]. Since mineralization occurs later along the fibrous network [39], the geometrical features play an important role in the bone regeneration process through their influence on the arrangement of the fibrous tissue.

It is important to realize that the time span during which tissue formation is monitored plays a potentially important in understanding the role of curvature and pore geometry on tissue regeneration. For example, it has been shown that bone tissue regeneration is a two-stage process with the initial stage, which takes up to a few weeks, being strongly dependent on the scaffold material rather than pore geometry. The second stage is, however, much more dependent on pore geometry than on the scaffold material [40]. The results of that study were later confirmed in a follow-up study [41] that showed up to four stages of tissue growth with geometry begin important only in the later stages. A short *in vitro* study may therefore be unable to capture the later stages of the tissue formation process and incorrectly conclude that geometry does not significantly influence the progress of the bone tissue regeneration process.

The role of inflammatory response in initiating the tissue repair and regeneration process has recently received attention. As noted by Almeida et al [42], regulating the right level of inflammatory response is emerging as a way of regulating the tissue repair and regeneration mechanism [43, 44]. Even though excessive inflammatory response may ultimately lead to implant rejection, moderate levels of inflammation may actually be helpful in triggering the tissue regeneration response of the human body [42]. Almedia et al [42] have shown that the scaffold geometry could influence the inflammatory response, with larger pore and wider angles resulting in secretion of higher levels of pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12/23, and IL-10 [42].

The shape of pores in porous scaffolds could vary anywhere between simple geometrical shapes such as cubic unit cells to more complex shapes such as rhombic dodecahedron and diamond-type unit cell [45, 46], and even advanced geometrical shapes such as minimal surfaces [47, 48]. Several studies have shown that pore shape could significantly influence the cell response and the rate of tissue regeneration [18, 20, 49, 50]. For example, Xu *et al.* compared square, triangular, and parallelogram pore shapes in an *in vitro* study [51] and

found that scaffolds with parallelogram pore geometry have the highest alkaline phosphate (ALP) activity among all the porous scaffolds with different pore geometries. However, it is not clear what exact geometrical feature gives rise to the elevated levels of ALP activity. It is therefore difficult to systemically study the effects of pore shape on the process of bone tissue regeneration. Some of the differences between different pore shapes are already captured by the studies that look into the effects of surface curvature and pore size on the performance of porous scaffolds. There may, however, be many other geometrical features that could potentially play a role in the process of bone tissue regeneration and are currently not identified. Identifying certain geometrical features that could potentially affect bone tissue regeneration requires studying different classes of pore shapes in a systematic way. Using a certain class of pore shape allows for isolating the effects of different geometrical features from each other and identifying new geometrical features that could potentially be useful for stimulating and guiding the process of bone tissue regeneration. No such studies are currently available in the literature.

The limitation of many of the studies performed so far is that the change in curvature is not isolated from the change in other potentially important parameters. For example, in some of the experiments, the examined curvature variations are associated with stiffness or pore size variations. It is therefore not clear to what extent the observed trends are a result of curvature differences.

2.3. Pore size and porosity

The effects of pore size on the bone tissue regeneration process have been studied since more than a decade ago. There is an excellent and well-known review paper by Karageorgiou and Kaplan that summarizes the findings regarding the effects of pore size on bone tissue regeneration up to 2005 [16]. Two most important findings of that review paper are that 1. the requirements regarding the pore size are different when comparing *in vitro* studies with *in*

vivo studies and that 2. pore sizes > 300 μm are recommended for scaffolds. As for the first finding, they found that lower porosity may be beneficial *in vitro* (Figure 4), because cell proliferation will be controlled and cell aggregation could be forced when the porosity is lower [16]. In comparison, higher porosity and larger pore sizes may be advantageous *in vivo*, because they could stimulate bone regeneration [16]. It is important to realize that the excellent review paper by Karageorgiou and Kaplan summarizes the studies performed up to 2005, where many of the currently available additive manufacturing techniques either did not exist or were not widespread. The studies reviewed in that article are therefore mostly performed using conventional manufacturing techniques. Conventional manufacturing techniques such as porogen leaching [52-54], phase separation [55-57], and gas foaming [28, 58, 59] have certain limitations that make it difficult for them to precisely control the pore size, porosity, interconnectivity, and pore shape of the porous scaffolds. It is particularly difficult to change one parameter while keeping the other parameters constant. For example, changing the pore size and porosity often may result in altered interconnectivity of the porous structure. It is therefore not easy to study the isolated effects of pore size using conventionally produced porous structures. Consequently, it is not clear to what extent the results of the previous studies regarding the pore size hold when the effects of pore size, pore shape, and interconnectivity are separated from each other. When using additive manufacturing techniques, one could precisely control pore size and pore shape while guaranteeing full interconnectivity of the porous structure. Even though studying the effects of pore size has continued after 2005 [60-65], the vast majority of the studies are still using the conventional production techniques. In one of the few studies that use additively manufactured porous scaffolds to look into the isolated effects of pore size on cell response and tissue regeneration, Van Bael et al [18] used selective laser melted porous scaffolds to show that a smaller pore size, i.e. 500 μm, improves cell response *in vitro* as compared to a

larger pore size of 1000 μm. Even when additive manufacturing techniques are used, it is not always easy to isolate the effects of pore size from other effects particularly from the effects of mechanical loading. It is known that mechanical loading partially controls bone apposition and plays an important role in bone tissue regeneration [66-69]. Changing the pore size while keeping the other parameters of the porous structure constant often results in altered mechanical properties of the porous scaffold [70, 71]. The change in the mechanical properties of the scaffold could potentially change the amount of mechanical load going through the scaffold (Figure 5) [72] and result in altered rate of bone tissue regeneration that is not caused by a different pore size but by a different share of mechanical load going through the porous scaffold (Figure 5). One way to circumvent this problem is to use different materials such that the large-scale mechanical properties of the porous scaffold remain the same regardless of different pore size. It is, however, important that the different materials do not have significantly different surface properties or topographies, because otherwise the effects of pore size will be mixed with those of surface (nano-) topography and surface chemistry.

3. MECHANISTIC ASPECTS

It is important to understand the mechanisms through which geometrical features interact with cells and influence the process of bone tissue regeneration. In this section, we will review the mechanistic aspects in the geometrical design of porous scaffold used for bone tissue regeneration purposes.

3.1. Pore shape and curvature

The most notable theories that explain the effects of pore shape and curvature on the process of bone tissue regeneration are based on mechanotransduction principles. The effects of geometrical features in general and curvature in particular have been studied at three scales: curvatures with radii much smaller than the cellular dimensions, curvatures with radii close to

the cellular dimensions, and curvatures with radii much larger than the cellular dimensions. When the radii of curvature are smaller than or comparable with the cellular dimensions, such as the case of nanotopographical features and nano-textures, theories that are based on modulation of focal adhesion [9, 73-75], contractile forces in the cytoskeletal network [76], and competition between shear stresses and the anisotropic bending stiffness of stress fibers [77] could explain the response of the cell to curvatures. Focal adhesion plays an important role when explaining the effects of curvature on the cell behavior. That is not surprising given the fact that focal adhesion sites are known to be instrumental in the pathways through which cells sense their surroundings [78]. Not only cells 'sense' physical forces through focal adhesion, they also 'apply' forces using pathways that involve focal adhesion [79]. That is why many types of interaction of cells with their physical environment including the interactions of cells with the geometrical features present on the surface of biomaterials involve focal adhesion.

Similar cellular and sub-cellular mechanisms are often used for describing the effects of geometrical features with radii of curvature larger than the cellular dimensions on tissue regeneration. Mathematical models of growth at larger length scales are required to link the consequences of those relatively large geometrical features to the sub-cellular phenomena such as focal adhesion, the stresses found in stress fibers, and other mechanotransduction pathways. Researchers have tried to use continuum growth theories [80-84] such as the one proposed by Ambrosi and co-workers [85, 86] to explain two main observations reviewed in the previous sections: 1. the rate of bone tissue regeneration increases with curvature and 2. bone tissue regeneration favors concave surface to convex and planar surfaces. In addition to the pioneering work of Rumpler et al [21], the most notable works in this regard are three consecutive papers from the same group [32, 33, 87] that have developed the theoretical basis for explaining both above-mentioned phenomena.

Rumpler et al. [21] postulated that the dependency of tissue growth on geometrical features is due to the mechanical forces that develop on various surfaces and could be very different from each other. For example, geometrical features with a higher curvature may result in higher levels of stress concentration [76], and, thus, higher levels of tissue regeneration stimulus. Through analogy with other phenomena such as membrane mechanics, crystal growth, and phase transformation [21], they postulated that surface tension may also be playing a role. The theoretical basis for explaining the role of surface tension was, however, not available back then. The theoretical basis required for explaining the relationship between curvature sign (convex vs. concave) was developed in two later studies [33, 87]. According to those studies [33, 87], the dependency of tissue growth on curvature sign could be explained by "the presence of contractile tensile stresses produced by cells near the tissue surface" [33]. Such surface stresses may prevent tissue from growing on convex surfaces [33]. It is important to realize that as tissue grows on the surface, layers of regenerated tissue may start to cover the convexities or flat surfaces and change the surface geometry locally, thereby enabling delayed growth of tissue on non-concave surfaces [21, 33]. The above-mentioned theoretical models were originally derived in two dimensions but were later extended to three dimensions [32], thereby enabling application of the developed theories for improvement of *in vitro* tissue regeneration.

It has been long known that geometrical features regulate cell alignment [88-90]. Most of the available studies have focused on the geometrical features that are present or could be created on the surface of biomaterials. In general, it has been shown that different types of cell align along the geometrical features of the surface such as grooves or fibers [30, 35, 90, 91]. Similar effects of surface topology on cell alignment have been observed for geometrical features ranging from micrometer to nanometer scales, although there seems to be a threshed above which the impact of geometrical features on cell alignment starts to be significant [90,

92]. A recent study has shown that cell alignment is more pronounced on curved surfaces and that smaller curvature results in stronger cell alignment [93]. It is therefore important to realize that such mechanisms as cell alignment might be playing certain (intermediate) roles in the cascade of events that regulate the effects of geometrical features such as curvature on the tissue regeneration process.

3.2. Pore size and porosity

The effects of pore size and porosity on bone tissue regeneration is a multi-faceted topic with several important mechanisms working simultaneously. First of all, it is important to distinguish between the effects of pore size and porosity on *in vitro* performance of cell culture constructs and *in vivo* bone regeneration performance of porous scaffolds [16]. Both the requirements regarding the pore size and porosity and the mechanisms that relate these parameters to their tissue regeneration systems could be very different to the extent that some of the effects could be completely opposite to each other. For example, the review of literature up to 2005 has shown that the effects of porosity on bone tissue regeneration is opposite when comparing *in vitro* and *in vivo* studies [16].

In general, pore size and porosity act through at least three different mechanisms. First, pore size and porosity regulate fluid flow and mass transport within the porous scaffold. Depending on the size of the pores and the porosity of the scaffold, the permeability of the porous scaffold and, thus, fluid velocity will be different [18]. *In vitro*, higher fluid velocity means that the cells have less time for attachment to the surface of the scaffold [18]. The cell seeding efficiency may therefore decrease with larger pores sizes [18]. *In vivo*, the transport of oxygen, nutrients, and waste through diffusion will be easier when pores sizes are larger [94]. That might be the reason why most *in vivo* studies show improved bone tissue regeneration with larger pores sizes [16]. As noted by Karageorgiou and Kaplan [16], pores size may also determine the route to bone regeneration: while smaller pore sizes result in

hypoxic conditions and chondrogenesis before ontogenesis, larger pore sizes result in direct ontogenesis and bone formation [95-97].

Second, pore size influences cell behavior through different mechanisms. For example, pores size and porosity could control cell proliferation and cell aggregation [16, 94]. It is not always clear whether such effects of pore size on cell behavior are independent from the effects of fluid flow and mass transfer or an indirect consequence of those mechanisms.

Third, higher porosity generally decreases the mechanical properties of the porous scaffold that could in turn result in altered state of mechanical loading *in vivo* [72]. Since bone apposition is dependent on mechanical loading [67, 98], different mechanical loading of the porous scaffold could potentially lead to altered states of *in vivo* bone tissue regeneration [71]. Additionally, too large of a pore size could compromise the structural integrity of the porous scaffold, meaning that it may not be capable of providing enough mechanical support particularly when used in load-bearing applications. That might impose an upper limit for the pore size particularly when porous scaffolds are made from non-metallic biomaterials. In the case of metallic biomaterials such as titanium and tantalum alloys, the mechanical properties of the solid matrix are so high that even very high porosities (e.g. 90%) and pore sizes may not pose a problem [45, 70].

It is important to realize that pore size and porosity are independent neither from each other nor from other geometrical features such as pore interconnectivity and surface curvature. Two distinct types of mixing are identifiable in the literature. The first type of mixing is not intrinsic, but merely a result of the limitations of the manufacturing technique. Since most of currently available studies have used conventional manufacturing techniques, the effects of different geometrical features are often mixed with each other in the vast majority of currently available studies [62]. This type of mixing of effects could be largely avoided when advanced additive manufacturing techniques are used. The second type of mixing is intrinsic

and not caused by the manufacturing techniques. For example, porosity and pore size are not completely independent. More importantly, there is an intrinsic connection between pore size and curvature. According to the Fenchel's theorem (1929) [99], the average curvature of any arbitrary but closed curve in space is $\geq 2\pi/P$ where P is the perimeter of the curve. The equality holds only for convex curves in the plane. Since average curvature is related to perimeter and perimeter is related to pore size, pore size and average curvature are intrinsically linked to each other. These types of relatively weak but intrinsic inter-dependencies make it more difficult to separate the effects of different geometrical parameters from each other.

One important point regarding the pore size is the effects of post-production treatments such as surface treatments on the pore size and mechanical properties of porous scaffolds. Depending on the specifications of different surface treatment protocols, it has been shown that they might have negligible [100, 101] or significant impact [100] on the pore size and the large-scale mechanical properties of porous scaffolds used for bone tissue substitution.

4. IMPLICATIONS FOR GEOMETRCIAL DESIGN OF SCAFFOLDS

The fact that geometrical features such as curvature and pore shape influence the bone tissue generation process calls for strategies using which the geometrical design of porous scaffolds could be optimized for best tissue regeneration performance. In this section, we will look into the implications of the above-mentioned observations for geometrical design of scaffolds.

4.1. Theoretical models and computational design tools

Optimizing the geometrical design of porous scaffolds would not be feasible without using computational models that could predict the tissue regeneration performance of different geometrical designs. Two types of theoretical models could be used in this regard. In the first type of computational models, tissue generation is directly linked to geometrical features such as curvature. In order to be able to establish such direct relationships, one needs to use

the phenomenological models that describe the relationship between geometrical features and tissue growth. For example, Guyot et al. [50] combined the phenomenological relationship between surface curvature and rate of tissue growth proposed by Rumpler et al. [21] with the level set method to study the effects of pore shape on the performance of porous scaffolds used for bone tissue regeneration. In this type of computational models, the link between growth dynamics and fundamental mechanical, chemical, and/or physical variables is not explicit and the accuracy of computational predictions is dependent on the accuracy of the phenomenological model used for relating geometrical features to growth dynamics.

In the second type of computational models, the tissue regeneration performance is linked to geometry through more fundamental quantities such as (surface) stresses. In this approach, a growth model is required to establish the link between geometrical features and those more fundamental quantities such as the state of (surface) stress. So far, continuum growth models have been used for establishing such links [32, 33, 87]. Such continuum growth models are suitable for studying the effects of mechanical variables such as stress and strain and linking them to growth dynamics. However, they cannot be used to study the effects of other parameters such as the transport of biological factors, oxygen, nutrients, and waste. In addition, continuum growth models are not suitable for combining the effects of mechanical factors with those of mass transport and chemical reactions to devise one single theoretical platform using which the geometrical design of scaffold could be optimized based on multiple design criteria. An alternative class of growth models, namely mixture models [81, 84, 102, 103], could be used to combine the effects of mechanical forces with those of chemical reactions, mass transport phenomena, and electrical potentials. Such a computational approach has not been followed before in the literature but could be rewarding as the effects of geometrical features and pores shape could be seen not only in the light of surface tension that they cause but also in terms of the permeability and the resulting

diffusion of different chemical species. This approach could therefore unify the design platform that is used for determining the pore size with the one used for deciding about the pore shape.

4.2. Geometry gradients

One concept that has not received much attention before is the concept of scaffolds with geometrical gradients. Since the geometrical requirements could be different at different stages of tissue regeneration, it has been suggested that porous scaffolds should designed with certain gradients in their geometrical features such as pore size and the type of curvature [16, 20]. No studies are currently available that explore the potential benefits of porous scaffolds with pre-designed gradients of geometrical features.

4.3. Minimal surfaces

One of biomimetic approaches in the design of porous scaffolds for bone tissue regeneration has been based on studying the structure of bone tissue and trying to replicate that in the design of porous scaffolds. As Bidan et al. [32] note, the average mean curvatures of trabecular bone is shown to be close to zero [104, 105]. This observation establishes a connection between the bone ultrastructure and the so-called minimal surfaces that are being studied by topologists as a research topic in the differential geometry of surfaces [106-108]. In brief, minimal surfaces locally minimize their surface energy, which is equivalent to having a mean curvature of zero [106-108]. Moreover, as previously discussed (section 3.1), the dependency of tissue growth on the sign of curvature has been linked to surface tensions [32]. It is therefore natural to consider minimal surfaces (Figure 6) for the design of bone tissue engineering scaffolds [47, 109]. In particular, a specific class of minimal surfaces, i.e. triply periodic minimal surfaces [48, 110, 111] (Figure 6) has been proposed for the design of porous scaffolds aimed for bone tissue regeneration applications. However, there is currently

not much experimental data to support the hypothesis that porous scaffolds designed based on minimal surfaces improve bone tissue regeneration.

5. DISCUSSION

The review of the literature thus far clearly shows that the geometrical deign of porous scaffolds significantly influences the bone tissue regeneration process. Geometrical design of porous scaffolds could therefore be used not only for supporting the bone tissue regeneration process but also for stimulating and guiding it. There are many geometrical features of the porous scaffolds that could potentially be important among which curvature and pore size have received the most attention so far. There are two major limitations concerning the currently available studies that need to be addressed in the future. First, the effects of different geometrical parameters are often mixed with each other. As previously mentioned, this is partly due to the fact that conventional manufacturing techniques are not capable of easily separating the different geometrical parameters such as pore size, pore shape, porosity, and interconnectivity of the porous structure. Recently developed additive manufacturing techniques could be helpful in separating the effects of different geometrical parameters from each other. However, there are certain intrinsic connections between the various geometrical parameters (section 3.2) that complicate separating the effects of different geometrical features even when additive manufacturing techniques such as selective laser melting [112-115] and electron beam melting [116-118] are used. In such conditions, full-factorial multi-parameter design of experiments as well as theoretical and computational models could play an important role in enabling the researchers to separate the effects of different interrelated parameters and reveal the interdependency of the different geometrical parameters both experimentally and numerically.

Another limitation of the currently available data is lack of sufficient *in vivo* and combined *in vitro/in vivo* data. The majority of the currently available studies use only *in vitro* assays for

analyzing the effects of geometrical features on cellular response and the bone tissue regeneration process. In the cases where *in vivo* data is available, the *in vitro* part is often missing. The availability of both *in vitro* and *in vivo* data together with computational (*in silico*) data is very important, because it could result in better understanding of the effects of geometrical features on the different steps of the bone regeneration process. As previously mentioned, the geometrical design requirements could be very different for *in vitro* and *in vivo* conditions and may even be opposite to each other [16]. Moreover, the effects of different mechanisms could be separated from each other when both *in vitro* and *in vivo* data is available. For example, the effects of modified mechanical loading caused by changes in the pore size and porosity are always present *in vivo*, while they could be avoided *in vitro*. Availability of both *in vitro* and *in vivo* data could therefore enable isolating the effects of mechanical loading from those of other parameters. *In silico* models could play a similar role as different types of mechanisms could be turned on or off in computer models to study whether a specific mechanism could explain the observed experimental data.

As previously mentioned, three specific mechanisms, namely mechanical loading, geometrical design, and substrate stiffness could influence the bone tissue regeneration process. There is, however, intrinsic interconnection between those mechanisms. For example, substrate stiffness is often related to the mechanical properties of the material from which the scaffold is made. The mechanical properties of the scaffold material also determine the mechanical properties of the porous scaffold at the homogenized, i.e. macro, scale. In turn, the mechanical properties of the porous scaffold determine the magnitude of the mechanical loading going through the scaffold, at least in a number of animal models used for evaluation of porous biomaterials [72]. There is therefore in many cases an intrinsic relationship between the substrate stiffness and mechanical loading going through the scaffold. The geometrical design of scaffolds is another factor that determines the mechanical properties of

porous scaffolds [45, 46]. Because of the above-mentioned connection between the mechanical properties and the mechanical loading going through the scaffold, the geometrical design of scaffolds is in many cases coupled with the mechanical loading going through the scaffold. Additive manufacturing techniques provide opportunities for decoupling these otherwise intimately coupled mechanisms. For example, one may be able to manufacture multi-material scaffolds where the material on the surface of the scaffold is different from the bulk of the matrix material and, thus, has different mechanical properties. This could decouple the effects of substrate stiffness from the effects of mechanical loading. For decoupling the effects of geometrical design from those of mechanical loading, one could manufacture dedicated porous structures using additive manufacturing techniques such that they are based on different geometrical designs but have identical macro-scale mechanical properties and, thus, identical mechanical loading.

Theoretical and computational models have been used to explain the effects of both curvature and pore size on the progress of the bone tissue regeneration process. As far as curvature is concerned, the theoretical models have been focusing on mechanical variables in the context of continuum growth theories. Such theoretical models are excellent first steps towards understanding the effects of curvature on the process of bone tissue regeneration. However, the links are missing on the one hand with chemical and mass transport phenomena and on the other hand with the subcellular and cytoskeletal phenomena. It is suggested that the models used for explaining the effects of curvature be expressed in the context of the mixture theory [81, 102, 103]. Mixture theory is an ideal platform for combining the effects of mechanical factors with those of chemical reactions and transport phenomena. It could therefore be used to combine the mechanical consequences of geometrical features with their consequence in terms of transport phenomena. Moreover, the theoretical models available at this larger scale need to be combined with the theoretical models that work at the smaller

scale and describe the consequences of topological and topographical features at the sub-cellular level [76, 77]. Such a combined theoretical basis could ultimately be used for optimal geometrical design of porous scaffolds aimed for bone tissue engineering applications.

Some new design concepts such as porous scaffolds with gradients of geometrical features and porous scaffolds based on minimal surfaces were also discussed in the previous sections. Both above-mentioned types of porous scaffolds could be realized using additive manufacturing techniques. It is suggested that the future studies focus on such newer design concepts and explore their potential benefits in terms of improved bone tissue generation performance of porous scaffolds.

6. CONCLUSIONS

The studies reviewed in this paper clearly show the importance of geometrical design of the scaffold on the process of bone tissue regeneration. In this context, both large geometrical features that are comparable in size with the cellular dimensions and geometrical features that are much smaller than the cellular dimensions play important roles. However, the primary focus of the current article was the importance of larger geometrical features. Among all possible geometrical features, curvature, pore size, pore shape, and porosity have received the most attention in the studies reviewed here. Regarding the curvature, it has been found that tissue regeneration occurs preferentially on concave surfaces and that the rate of tissue regeneration increases almost linearly with curvature. As for pore size, the current evidence shows that a minimum pore size in the order of a few hundred micrometers is necessary for successful bone tissue regeneration. In the case of porosity, *in vitro* and *in vivo* studies suggest contrary effects of porosity on bone tissue regeneration. While higher porosity results in improved bone regeneration *in vivo*, smaller porosity is found to be more effective *in vitro*. This finding challenges the use of *in vitro* assays for examining the effects of geometrical design of scaffolds on the performance of the bone tissue regeneration process. An important

limitation of many studies reviewed in this article is that different geometrical features were not always changed independently from each other. That is primarily due to the limitation of conventional manufacturing processes used for fabrication of scaffolds. The recent advances in additive manufacturing techniques have created opportunities to investigate the isolated effects of different geometrical features. It is suggested that future studies use this new opportunity to study the isolated effects of several rigorously defined geometrical features on the performance of the bone tissue regeneration process.

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Figure captions

Figure 1. An example of porous titanium implants manufactured using an additive manufacturing technique, namely selective laser melting, and aimed for treatment of 6 mm long segmental bone defects in a rat animal model [72]. The porous structure is based on dodecahedron unit cells. (Reprinted from Journal of Biomechanics, 47, S. Amin Yavari, J. van der Stok, S.M. Ahmadi, R. Wauthle, J. Schrooten, H. Weinans, A.A. Zadpoor, Mechanical analysis of a rodent segmental bone defect model: The effects of internal fixation and implant stiffness on load transfer, pp 2700–2708, Copyright 2014, with permission from Elsevier).

Figure 2. Staining of actin stress fibers to visualize the tissue formed *in vitro* and to study the effects of curvature [21] (a). The predicted tissue regeneration based on a linear curvature-dependent theoretical model [21] is depicted in subfigure (b). Theoretical predictions match these *in vitro* experimental observations.

Figure 3. Blood vessels are generated in the concavities on the surface of an implant [37] (a-b) (reproduced from Figure 5 of reference [37] with kind permission from Springer Science and Business Media). Similarly, *in vitro* results show much larger volumes of generated tissue on concave surfaces as compared to convex surfaces [33] (c) (Reprinted from Acta Biomaterialia, 9, E. Gamsjäger, C.M. Bidan, F.D. Fischer, P. Fratzl, J.W.C. Dunlop, Modelling the role of surface stress on the kinetics of tissue growth in confined geometries, pp 5531–5543, Copyright 2013, with permission from Elsevier). The distribution of both actin fibers and myosin lib is dependent on local curvature (d) [19]. The density of both increases on concave surface, suggesting locally higher states of cell stress (d) [19].

Figure 4. *In vitro*, the pore-filling behavior of fibroblasts is dependent on the pore size (small, medium, or large) [119]. The pore filling tends to be more complete for smaller pore sizes.

Figure 5. An *ex vivo* picture of a segmental bone defect model [72] (a). In this model, the share of the load going through the implant is deponent on the stiffness of the implant [120] (b). Since changing the porosity of the porous structure drastically changes the mechanical properties of the porous structure, it could also change the mechanical loading going through the implant and influence the process of bone tissue regeneration. (Reprinted from Journal of Biomechanics, 47, S. Amin Yavari, J. van der Stok, S.M. Ahmadi, R. Wauthle, J. Schrooten,H. Weinans, A.A. Zadpoor, Mechanical analysis of a rodent segmental bone defect model: The effects of internal fixation and implant stiffness on load transfer, pp 2700–2708, Copyright 2014, with permission from Elsevier).

Figure 6. Some examples of the possible unit cells for creating porous scaffolds based on triply periodic minimal surfaces. The example unit cells include primitive (a), diamond (b), gyroid (c), and I-WP (d) minimal surfaces.

Figure 1

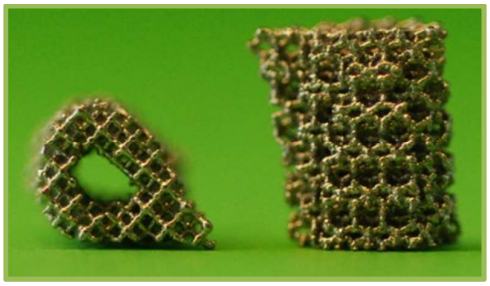


Figure 2

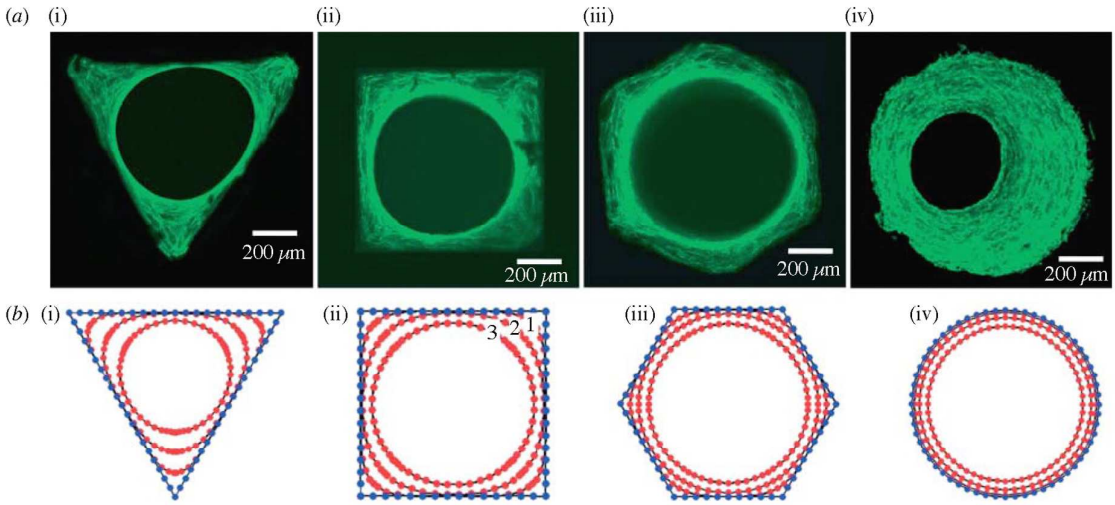
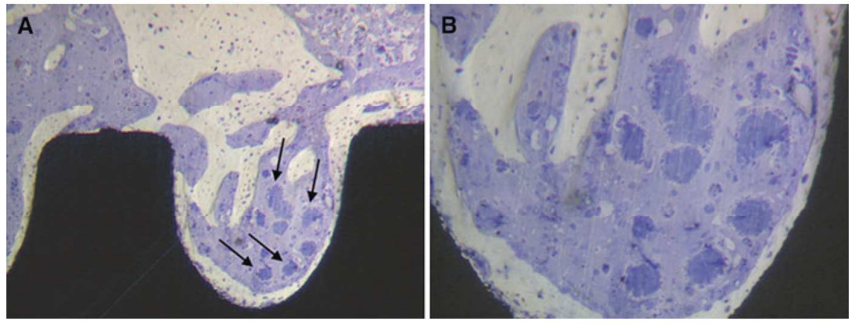
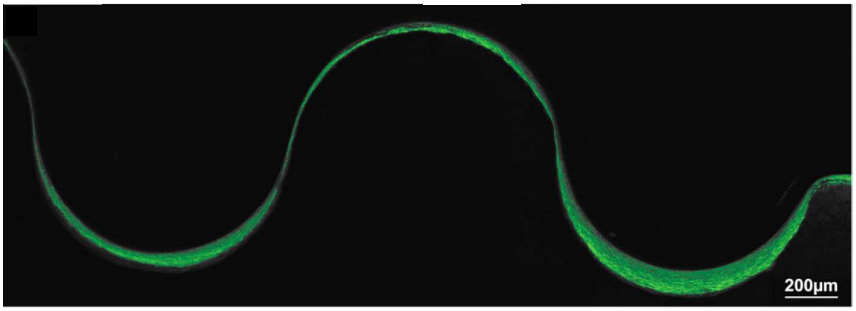


Figure 3



a) **b)**



c)

d)

Figure 4

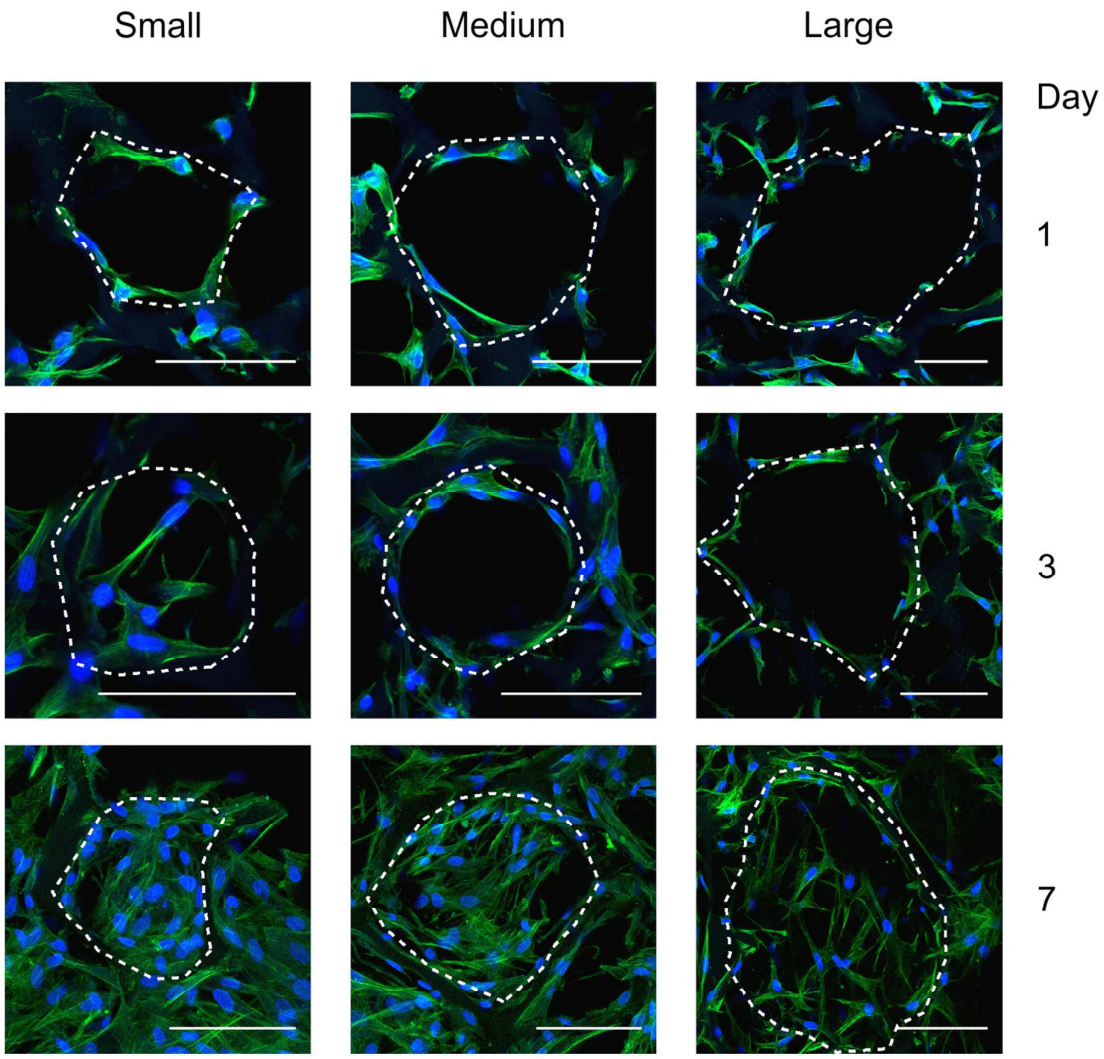


Figure 5

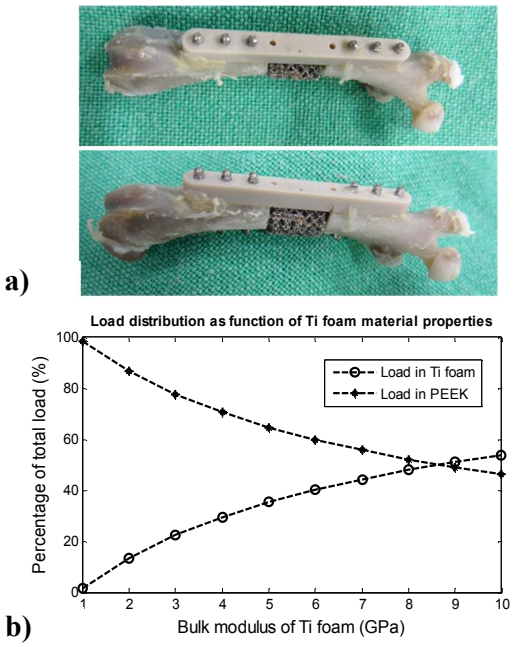
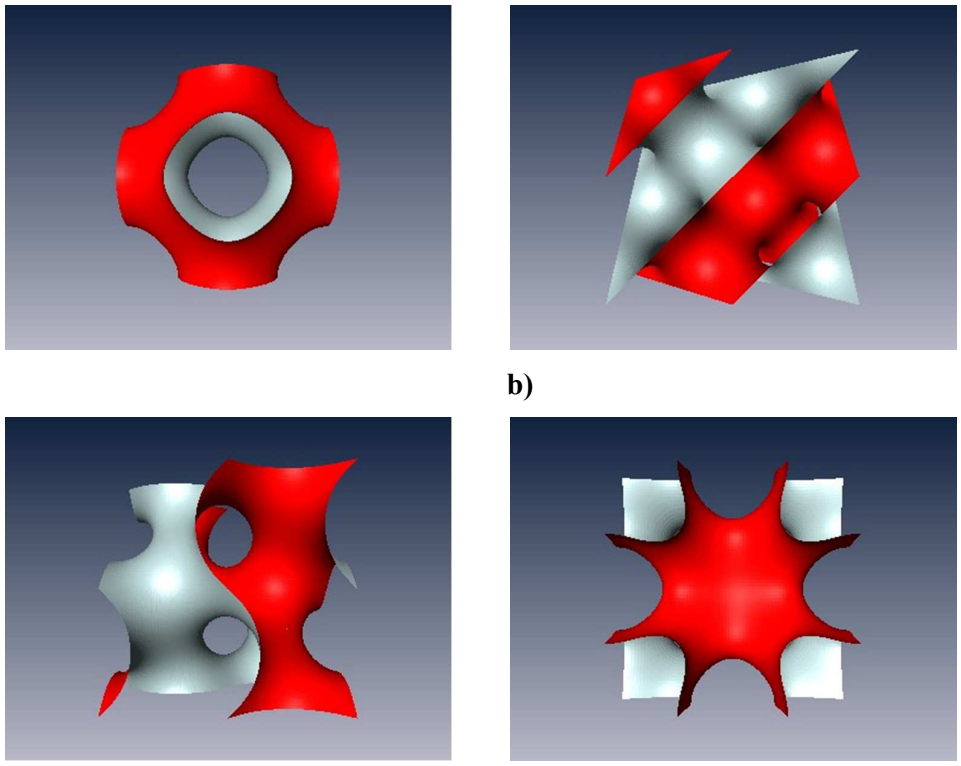

a)
b)
Load distribution as function of Ti foam material properties
Load in Ti foam
Load in PEEK
Percentage of total load (%)
Bulk modulus of Ti foam (GPa)
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10

Figure 6



a) **b)**

c) **d)**