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Commentary

Commentary: Deciphering the link between architecture and biological response of a bone graft substitute

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ABSTRACT

Hundreds of studies have been devoted to the search for the ideal architecture for bone scaffold. Despite these efforts, results are often contradictory, and rules derived from these studies are accordingly vague. In fact, there is enough evidence to postulate that ideal scaffold architecture does not exist. The aim of this document is to explain this statement and review new approaches to decipher the existing but complex link between scaffold architecture and *in vivo* response.

1. Introduction

A few million people undergo bone grafting procedures every year. The aim of the graft, either natural or artificial, is to act as a scaffold to which cells to migrate, proliferate, differentiate and synthesize new bone. To optimize these tasks, the scaffold must be biodegradable and preferably porous. Indeed, biodegradability is mandatory to obtain full conversion of the scaffold into mature and mechanically viable bone, and the presence of pores accelerates this process. Therefore, numerous studies have been devoted to the quest for an optimal scaffold design. Despite these efforts, it is still not clear what the best scaffold architecture (pore size, shape, interconnectivity) should be. For example, Karageorgiou and Kaplan [1], who recently reviewed the topic thoroughly, could only draw very vague conclusions, such as "pore sizes >300 μm are recommended". In other words, the increment of knowledge that has been gained since the first publications on the topic by Klawitter and Hulbert [2,3] 40 years ago, who advised that scaffolds should be porous and pores be interconnected with a minimum interconnection size of 100 μm, is very limited. A possible explanation for the absence of clear findings about scaffold architecture is that there is simply no optimum scaffold architecture. This somewhat provocative statement is in fact supported by a number of experimental results. The main aim of this document is to explore this statement in more detail. The absence of an optimum does not mean that scaffold architecture does not affect the biological response. Numerous studies have indeed shown that significant differences exist between various architectures. However, since the interactions between cells and a porous and biodegradable scaffold are very complex, it has been difficult to draw general conclusions. Therefore, this study also aims to present new approaches to deciphering the relation between scaffold architecture and biological response more easily. This commentary is devoted to bone, but the points discussed are likely to be applicable to other organs. The document is divided into two main parts: Section 2 describes the difficulties related to the study of the interplay between porous materials and biological systems; Section 3 describes a systematic and scientific approach to studying this interplay more effectively.

2. Difficulties in studying the interplay between porous materials and bone

Four main observations may help to illustrate the complexity of researching an optimal pore size. First, scaffolds should fulfill not only one, but many functions (or tasks) before, during and after implantation. Second, finding an optimum architecture implies that it is possible to characterize a porous network precisely and also design the envisioned scaffold precisely, which is generally not feasible. Third, the use of biodegradable scaffolds is correlated with two major difficulties: the scaffold architecture changes with the degradation process, and the degradation by-products affect

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the biological response. Fourth, the biological response of a material depends not only on the material properties (e.g., solubility, scaffold architecture), and its interactions with the biological environment (e.g., protein adsorption, cell adhesion, mechano-transduction), but also on patient-related aspects such as age, gender and genetics. The aim of the following sub-sections is to address these four aspects.

2.1. Multiple functions

Defining the most adequate scaffold architecture of a bone scaffold for bone application is in fact an attempt to find a compromise between various conflicting functional requirements. For example, increased mechanical function requires a dense scaffold, while enhanced cell/gene delivery requires a porous scaffold [4]. Similarly, high permeability favors nutrient transport, but may surprisingly decrease the ability of a scaffold to be impregnated with a fluid (e.g., blood) prior to implantation [5]. There is also evidence that scaffold resorption is enhanced by small pores and a high surface area [6], whereas pore sizes >300 μm are recommended for bone in-growth [1]. In other words, determining the best scaffold architecture is a relative concept, because a true optimum can probably only be found for one specific function. In that respect, it should be stated that most scientific studies do not clearly identify which function they aim to optimize, and which function(s) they diminish as a consequence of this "optimization".

2.2. Scaffold characterization and design

Numerous methods can be used to analyze pores: for example, optical approaches (microscopy), physico-chemical approaches (nitrogen adsorption and desorption) and capillary approaches (mercury porosimetry) [7]. However, only advanced medical imaging techniques such as micro-computed tomography (μCT) [7,8] and magnetic resonance imaging [9] can provide a three-dimensional (3D) representation of the scaffold. Although these techniques are extremely useful and powerful, they have limitations, such as the difficulty to process the large amount of collected data. Also, high resolutions can only be obtained *ex vivo* or *in vitro* and not *in vivo*, meaning that *in vivo* monitoring is not possible for large animals and is also questionable with regard to the large X-ray doses applied to animals.

Provided (i) it is possible to scan the scaffold architecture with an adequate resolution and (ii) the collected data can be analyzed in a reasonable time, difficulty arises when trying to define the architecture, e.g., as a function of pore size and shape. Indeed, a mean pore size can be expressed in different ways, e.g., as a function of pore number, pore surface or pore volume. Also, a pore generally has a more complex geometry than a sphere or a cube. In that case, authors use either a discrete or a continuous approach to determine the pore size [10]. In the discrete approach, each pore is approximated with one single value, for instance the size of the largest sphere that can be fitted within the given pore space (Fig. 1). In the continuous approach, each pore is simulated with a pore size distribution, e.g., by relating the diameter of a sphere to the volume fraction of the pore that can be filled by this sphere. Obviously, a smaller sphere can fill up a larger portion of the pore space. The continuous approach delivers smaller mean pore sizes than the discrete approach does. Another important point is that the determination of the mean pore size is based on the assumption that the pores have a specific geometry (spheres, for instance), which is obviously a crude approximation when a discrete approach is used to define pore size (Fig. 1). The already complex problem of defining pore geometry is hindered by the fact that scaffolds should contain not only single pores, but also interconnected pores to allow cell in-growth and nutrient transport. Moreover, these pores (generally referred to as "macropores") and pore interconnections should be larger than ~50 μm to provide enough space for in-growing blood vessels [1,11,12]. So, not only the pores, but also the pore interconnections must be characterized (Fig. 1). In addition, since the velocity of fluid and nutrient transport is highly dependent on the direction of fluid supply, it is likely that the relative orientation of the pores and their interconnections is critical for bone/scaffold interactions. Recent studies suggest also that micropores (diameter typically in the range 0.1–10 μm) may have a very large effect on the *in vivo* response [13–15], and hence their shape and size would also have to be characterized. In summary, it is possible neither to define perfectly the geometry of most scaffolds used as bone graft substitute, nor to compare the results of various studies, because the method used to determine the pore and interconnection size varies from study to study.

Provided one is able to define the ideal scaffold architecture, an additional problem may arise when trying to test it *in vivo*, because not all scaffold architectures can be manufactured. Despite some exceptions (e.g., 2-photon lithography [16] and robocasting [17]), the resolution of solid free-form fabrication (SFFF) approaches is generally limited to a few dozen micrometers [18], whereas the so-called micropores, which are generally defined in the biomaterials field as pores with size in the range 0.1 to 10–20 μm, have been suggested to be essential for scaffold degradation [13] and nutrient transport [19].

2.3. Biodegradation and pore size

Nowadays, the concept of "regenerative medicine" is widespread. To apply this concept in the field of bone requires the use of biodegradable materials, such as polylactides, collagen or calcium phosphates, which substantially complicates the search for the most adequate scaffold architecture. First, the scaffold architecture of these materials varies during degradation. Second, the *in vivo* degradation of the scaffold decreases the mechanical properties over time, which may affect the biological response. Third, degradation generates by-products which may affect the biological response. For example, Ignatius et al. [20] observed strong inflammatory reactions 2 years after implanting calcium phosphate–polylactides composites in sheep. The biological response may also be positive, since many ions, such as calcium and phosphate ions, are known to trigger biological responses [21–23]. So, biodegradation products must be considered when designing bone scaffolds. Efforts have been made in this direction recently [24], but this concept is still widely overlooked. The third problem raised by the use of biodegradable scaffolds is that the volume of the bone graft substitute is likely to influence the local release of degradation products, hence implying that the best scaffold architecture is a function of scaffold size, as predicted in a recent study [6].

2.4. Material- and patient-related aspects

So far, only a few scaffold properties have been considered, such as scaffold architecture and biodegradation, but there are many other parameters influencing the interplay between an implanted material and a biological system (Fig. 2). For example, material degradation is affected by a change in composition [25], microporosity [13], crystal size, crystallinity and surface properties [26]. Also, the biological response strongly depends on the patient age [27], metabolism [28], gender [29] or addictions [30,31], as well as implant loading [32], implant location [25] and cell–material interactions (protein adhesion, cell adhesion, cell proliferation). Since the biodegradation of a bone graft substitute may trigger biological reactions, any change in local metabolism will obviously modify the biological response and, as a result, modify the requirements set for the most ideal scaffold architecture. Another impor-

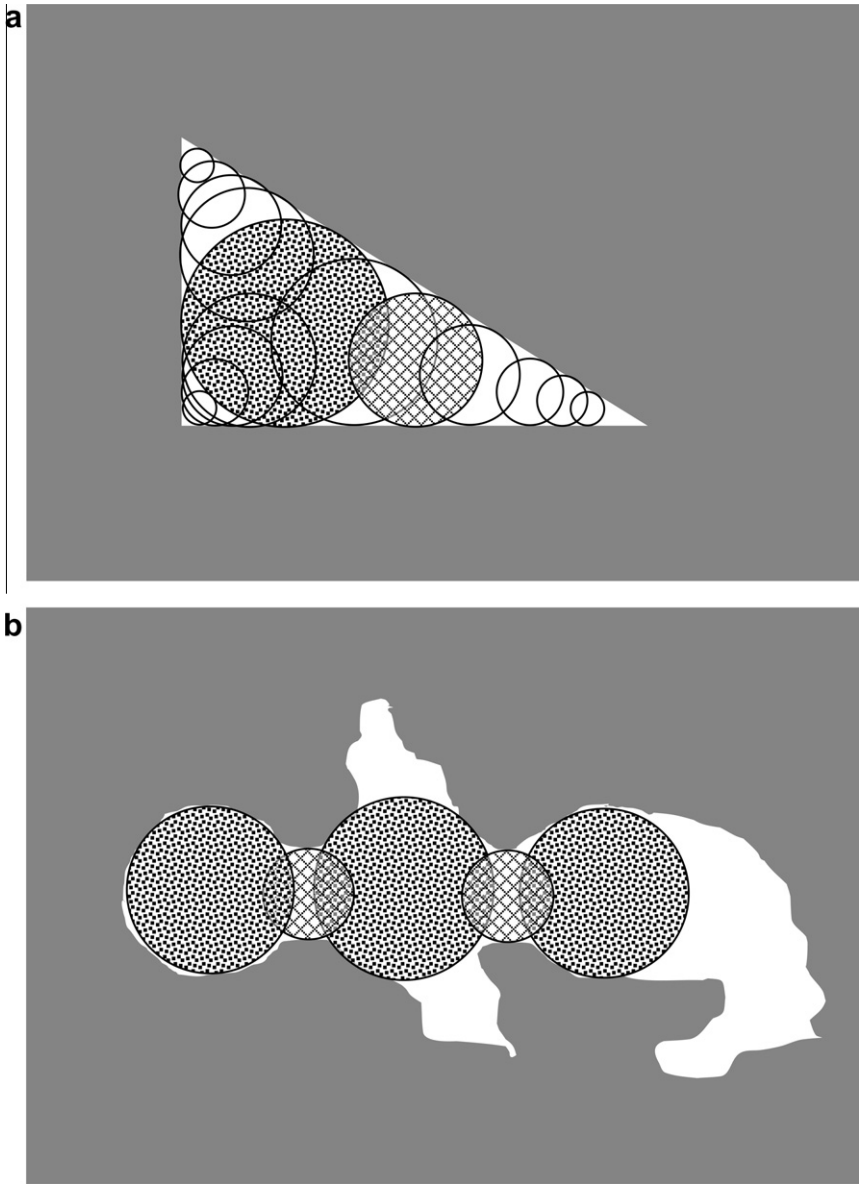

Fig. 1. Two-dimensional representation of how the pore and interconnection size of a scaffold are generally determined. In the continuous approach, the size of a given pore is generally approximated by the mean volume of all spheres that can be fitted into the pore (checked sphere in (a)). In the discrete approach, the size of a pore is generally approximated by the volume of the largest sphere that can be fitted into the pore (dotted sphere in (a)). (b) Shows several pores of identical size according to the continuous approach (checked spheres). The size of the pore interconnection is generally defined as the size of the smallest sphere that can be fitted between two pores (dotted spheres). In other words, an interconnection size corresponds to a local size minimum, whereas the pore size corresponds to a local size maximum. This scheme shows the limitations in describing the size of a pore, particularly for complex forms.

tant point to mention is that biological systems behave stochastically (non-deterministically) in the eyes of engineers, i.e., optimization studies are very difficult to perform owing to the inherent variability of the biological response. As a result, many different scaffold architectures might perform extremely well, even though none of them is the optimal scaffold architecture for the studied function.

The previous paragraphs have addressed the difficulties in defining an optimum scaffold architecture because: (i) each scaffold must fulfill several functions, such as resorption, bone ingrowth or mechanical support; (ii) it is difficult to characterize an architecture technically and mathematically; (iii) biodegradation products affect the biological response; and (iv) biodegradation depends on the material composition and solubility, as well as the patient and location of the scaffold. All these difficulties suggest that an ideal scaffold architecture does not exist. That would be the easiest explanation for the contradictory results presented in the literature. It would also imply that the design of studies devoted to the existing interplay between scaffold architecture and biological systems should be approached differently. The aim of the second part of this document is to describe how the authors consider this problem, and to discuss some of the newest approaches in the field.

3. New approaches to deciphering the link between scaffold architecture and biological response

To decipher the link between scaffold architecture and biological response, the various factors affecting biological response should be identified and their effects and interactions understood. If the effect of each factor or combination of factors were under-

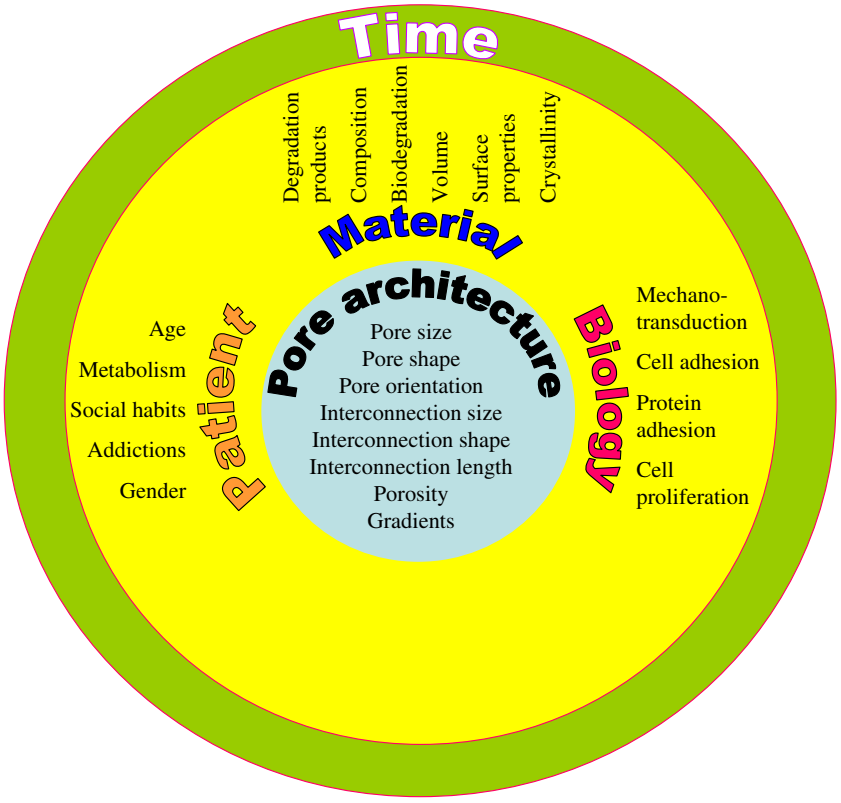

Fig. 2. Complex interplay between factors related to the patient, the material and the cells present at the material surface.

stood, it would be possible to design more adequate scaffolds. Unfortunately, there is limited knowledge in this field at present. This situation could be improved by designing *in vivo* studies according to six steps (Fig. 3): (i) propose a model to explain the effect of one specific feature (or combination of features) of a scaffold on the interaction between scaffold and biological systems; (ii) carefully design scaffolds to test the proposed model; (iii) extensively characterize the scaffolds prior to implantation; (iv) perform the *in vivo* study; (v) analyze the results of the *in vivo* study; (vi) validate the model based on the *in vivo* data. The following subsections detail these six steps.

3.1. Models for in vivo response

Hundreds of studies have been performed with the aim of determining the optimum architecture (generally pore size or pore interconnection size) for a scaffold. With only a few exceptions, studies have all been descriptive or exploratory: scaffolds with various architectures were produced, implanted, and their *in vivo* response was analyzed. In most cases, the architecture of the implanted scaffolds was only partially characterized, and no attempt was made to model the results. In the authors' opinion, it is more productive to think first about a way to model the *in vivo* response of a scaffold and then try to design a study to test the model. For example, one might want to correlate the relationship between the geometry and the resorption of a scaffold, independently of its composition [6]. Another goal could be to find the link between interconnection length and *in vivo* response [12].

3.2. Scaffold design

Once a model has been proposed, the scaffolds should be carefully designed. More specifically, only the factor of interest (e.g., interconnection length) should vary. This is not always possible, but the development of advanced manufacturing techniques, such as SFFF techniques [4,18], considerably eases this task. In certain cases, finite-element (FE) analysis may also be helpful. For example, Lin et al. [33] proposed a computer tool to design scaffolds with controlled porosity and elastic properties. These authors then produced the scaffolds by SFFF and validated their predictions. A similar approach was used by Hollister [18] to produce craniofacial scaffolds with controlled permeability and elastic properties. For more details, the readers are advised to read a recent review on the topic [34].

3.3. Scaffold characterization prior to implantation

After their production, scaffolds should be adequately characterized. Scaffold characterization should focus not only on the scaffold geometry such as size and shape of pores and pore interconnections or surface topography, but also on the physico-chemical properties such as solubility, molecular weight or crystal size. In terms of 3D imaging, large advances have been made in recent years [7], in particular with μCT [8], but it is still very difficult to obtain a precise description of large samples (>1 cm^3) at a submicrometer level. Since there is a correlation between scaffold degradation and *in vivo* response, the physico-chemical properties of the scaffolds should also be investigated prior to implantation.

3.4. In vivo study

After thorough characterization, the samples must be implanted, and their *in vivo* response must be monitored. Unfortunately, the tools that can be used to study the *in vivo* evolution of the scaffold are too limited at present, so the assessment of the *in vivo* performance is generally performed *a posteriori*. How-

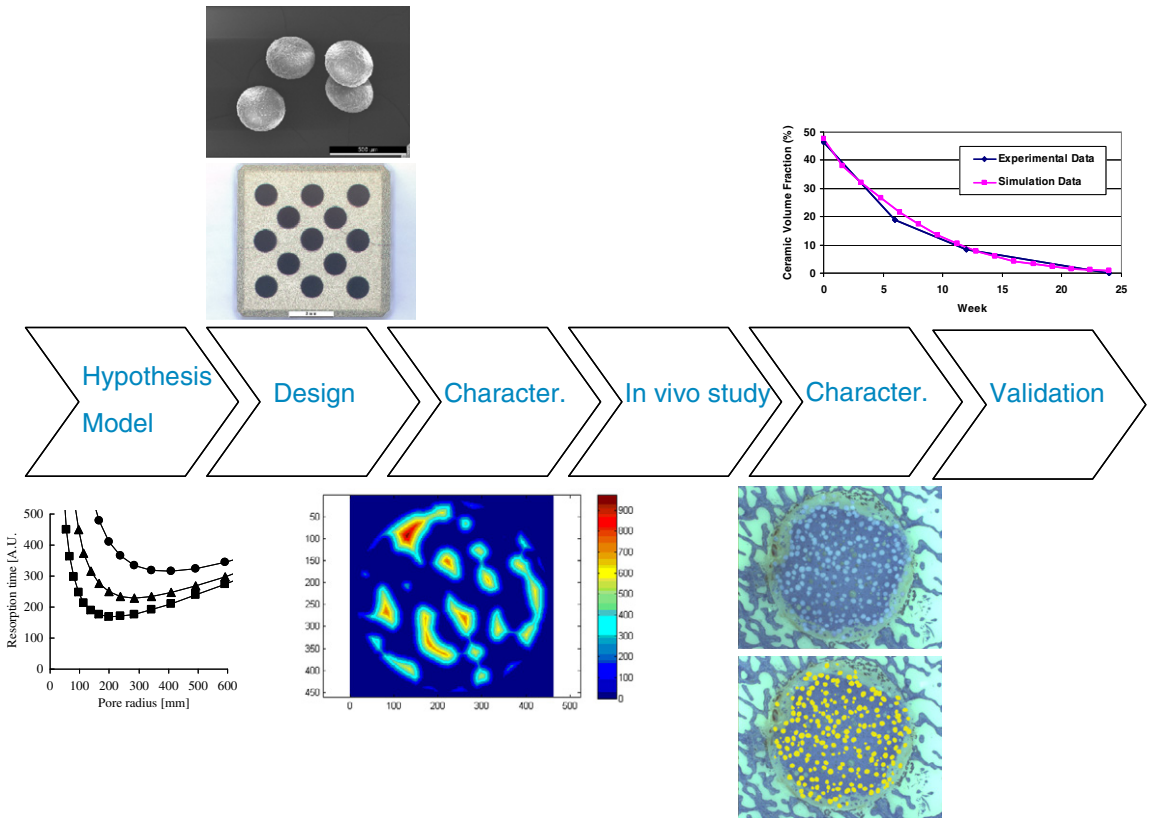

Fig. 3. The six steps involved in project design: (i) propose a model to explain the effect of one specific feature (or combination of features) of a scaffold on the interaction between scaffold and biological systems; (ii) carefully design scaffolds to test the proposed model; (iii) extensively characterize the scaffolds prior to implantation; (iv) perform the *in vivo* study; (v) analyze the results of the *in vivo* study; (vi) validate the model based on the *in vivo* data. Photographs are added to illustrate the various steps.

ever, there is a clear trend towards this goal, and first *in vitro* and *in vivo* results have been reported [35,36].

3.5. Scaffold characterization during and after implantation

Once retrieved, the implanted samples must be carefully characterized in terms of their physico-chemical properties, geometrical features and biological response. Regarding 3D imaging, exciting progress has been made in recent years [37,38]. For example, blood vessels can be detected using synchrotron light and pseudo-holotomography [39]. Similarly, zones of new mineralization can be observed at a very early stage [40]. Furthermore, the possibility to align two images obtained from different angles allows a comparison of scaffolds before and after implantation [41]. At present, limited resolution and large amounts of data are a bottleneck retarding further progress. However, it is likely that these aspects will be much improved in the near future.

Investigation of the biological response has also been eased in recent years, e.g., by the introduction of embedding resin hardening in cold conditions. With this new approach, it is possible to perform enzyme histochemistry, immunohistochemistry, a great variety of classical histological stains and even in situ hybridization on hard tissue–implant interfaces [42].

3.6. Model validation

Once *in vivo* data have been collected, the results must be analyzed in detail. Ideally, this should imply the application of a phenomenological model to the data. For example, Sandino et al. [19] looked at the link between scaffold permeability, mechanical stimulation and the type of tissues (bone-like, fibrous, cartilage-like) present within the pores. The same group applied a stochastic biophysical model to look at osteogenesis [43]. Unfortunately, there is a strong need at present for models describing the interaction between single cells and scaffold or extracellular matrix [44,45]. Also, the application of a mathematical model on complex structures requires very large computer capabilities, particularly if the results should be followed over time. One common approach to reducing this problem is to assume that one part of the scaffold, e.g., one thin slice of a cubic scaffold, is representative of the behavior of the whole scaffold. As a result, calculations must only be performed on this small slice. However, this approach can only be applied if the pores have identical size and shape, and if the scaffold symmetry allows it (e.g., radial geometry when considering a cylinder). So, as for 3D characterization, there is a need for improved computer power in order for biomaterial scientists to take full advantage of the proposed tools.

Nevertheless, the general approach proposed in this document has proved to be successful. In 2003, a simple model describing the effect of scaffold macro architecture (or geometry) and cell-mediated resorption of scaffolds was proposed and applied to already-published data [6]. In 9 of 12 cases, the correlation coefficient r^2 between the model and *in vivo* data was >0.90. These preliminary results suggest that the model can be used to design scaffolds with high resorption rates or to calculate the resorption rate of scaffolds, independently of the geometry.

The previous sections have proposed an approach to improve the yield of *in vivo* studies attempting to decipher the link between scaffold architecture and *in vivo* response. The approach is in itself quite obvious and simple, but its application is utterly complex. One way to solve this problem could be to create a taskforce of interdisciplinary experts working together to design, perform and

analyze the results of *in vivo* studies. A more sensible and pragmatic solution could be to ask authors of new *in vivo* studies to perform extensive characterizations of their scaffolds (material and architecture) according to unified and standardized methods. Also, free access to the data should probably be granted to other researchers, hence enabling other teams to test new aspects not yet addressed.

To advocate strong use of numerical approaches is not an attempt to discredit or to replace *in vivo* studies. In fact, numerical approaches are tools to maximize the output of biological studies [46]. For example, most *in vivo* studies look at the biological response of a bone scaffold at a macroscopic level, whereas the combination of 3D imaging techniques and FE analysis allows a local or microscopic approach: each and every location of the scaffold can be analyzed separately. As a result, the output of each study is greatly improved [39–41]. The use of 3D imaging techniques combined with FE analysis also has another advantage compared with other characterization techniques: it is possible to calculate the mechanical deformations at the scaffold surface or the fluid flow and nutrient transport within the scaffold [47]. Using biophysical models, a correlation between cell behavior and physical stimuli should be possible. Once such a model is established (or validated), it is possible systematically to investigate the effect of one or several parameters on the biological response.

4. Conclusion

The first part of this document highlighted the difficulties in defining an optimum scaffold architecture because: (i) each scaffold must fulfill several functions such as resorption, bone ingrowth or mechanical support; (ii) it is difficult to characterize an architecture technically and mathematically; (iii) biodegradation products affect the biological response; and (iv) biodegradation depends on the material composition and solubility, as well as the patient and location of the scaffold. All these difficulties suggest that an ideal scaffold architecture does not exist.

Nevertheless, there is a link between scaffold architecture and *in vivo* response. Unfortunately, this interplay remains mostly obscure. So a new approach was proposed in the second part of this document. This new approach is based on six steps: (i) propose a model to explain the effect of one specific feature (or combination of features) of a scaffold on the interaction between scaffold and biological systems; (ii) carefully design scaffolds to test the proposed model; (iii) extensively characterize the scaffolds prior to implantation; (iv) perform the *in vivo* study; (v) analyze the results of the *in vivo* study; (vi) validate the model based on the *in vivo* data.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 2 and 3 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2010.08.008.

References

[1] Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26(27):5474–91.
[2] Hulbert SF, Young FA, Mathews RS, Klawitter JJ, Talbert CD, Stelling FH. Potential of ceramic materials as permanently implantable skeletal prostheses. J Biomed Mater Res 1970;4(3):433–56.
[3] Klawitter JJ, Hulbert SF. Application of porous ceramics for the attachment of load bearing internal orthopedic applications. J Biomed Mater Res Symp 1971;2(1):161–229.
[4] Hollister SJ, Maddox RD, Taboas JM. Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. Biomaterials 2002;23(20):4095–103.
[5] Stähli C, Bohner M, Bashoor-Zadeh M, Doebelin N, Baroud G. Aqueous impregnation of porous β-tricalcium phosphate scaffolds. Acta Biomater 2010;6(6):2760–72.
[6] Bohner M, Baumgart F. Theoretical model to determine the effects of geometrical factors on the resorption of calcium phosphate bone substitutes. Biomaterials 2004;25(17):3569–82.
[7] Ho ST, Hutmacher DW. A comparison of micro CT with other techniques used in the characterization of scaffolds. Biomaterials 2006;27(8):1362–76.
[8] van Lenthe GH, Hagenmuller H, Bohner M, Hollister SJ, Meinel L, Muller R. Nondestructive micro-computed tomography for biological imaging and quantification of scaffold-bone interaction in vivo. Biomaterials 2007;28(15):2479–90.
[9] Potter K, Sweet DE, Anderson P, Davis GR, Isogai N, Asamura S, et al. Non-destructive studies of tissue-engineered phalanges by magnetic resonance microscopy and X-ray microtomography. Bone 2006;38(3):350–8.
[10] Muench B, Holzer L. Contradicting geometrical concepts in pore size analysis attained with electron microscopy and mercury intrusion. J Am Ceram Soc 2008;91(12):4059–67.
[11] Lu JX, Flautre B, Anselme K, Hardouin P, Gallur A, Descamps M, et al. Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo. J Mater Sci Mater Med 1999;10(2):111–20.
[12] Otsuki B, Takemoto M, Fujibayashi S, Neo M, Kokubo T, Nakamura T. Pore throat size and connectivity determine bone and tissue ingrowth into porous implants: three-dimensional micro-CT based structural analyses of porous bioactive titanium implants. Biomaterials 2006;27(35):5892–900.
[13] Klein CP, de Groot K, Driessen AA, van der Lubbe HB. Interaction of biodegradable beta-whitlockite ceramics with bone tissue: an in vivo study. Biomaterials 1985;6(3):189–92.
[14] Lan Levengood SK, Polak SJ, Wheeler MB, Maki AJ, Clark SG, Jamison RD, et al. Multiscale osteointegration as a new paradigm for the design of calcium phosphate scaffolds for bone regeneration. Biomaterials 2010;31(13):3552–63.
[15] Simon JL, Michna S, Lewis JA, Rekow ED, Thompson VP, Smay JE, et al. In vivo bone response to 3D periodic hydroxyapatite scaffolds assembled by direct ink writing. J Biomed Mater Res A 2007;83(3):747–58.
[16] Liska R, Schuster M, Inführ R, Turecek C, Fritscher C, Seidl B, et al. Photopolymers for rapid prototyping. J Coatings Tech Res 2007;4(4):505–10.
[17] Gratson GM, Xu M, Lewis JA. Direct writing of three-dimensional webs. Nature 2004;428(6981):386.
[18] Hollister SJ. Porous scaffold design for tissue engineering. Nat Mater 2005;4(7):518–24.
[19] Sandino C, Checa S, Prendergast PJ, Lacroix D. Simulation of angiogenesis and cell differentiation in a CaP scaffold subjected to compressive strains using a lattice modeling approach. Biomaterials 2010;31(8):2446–52.
[20] Ignatius AA, Betz O, Augat P, Claes LE. In vivo investigations on composites made of resorbable ceramics and poly(lactide) used as bone graft substitutes. J Biomed Mater Res 2001;58(6):701–9.
[21] Meleti Z, Shapiro IM, Adams CS. Inorganic phosphate induces apoptosis of osteoblast-like cells in culture. Bone 2000;27(3):359–66.
[22] Wu X, Itoh N, Taniguchi T, Nakanishi T, Tanaka K. Requirement of calcium and phosphate ions in expression of sodium-dependent vitamin C transporter 2 and osteopontin in MC3T3-E1 osteoblastic cells. Biochim Biophys Acta – Mol Cell Res 2003;1641(1):65–70.
[23] Zaidi M, Datta HK, Patchell A, Moonga B, MacIntyre I. 'Calcium-activated' intracellular calcium elevation: a novel mechanism of osteoclast regulation. Biochem Biophys Res Commun 1989;163(3):1461–5.
[24] Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol 2005;23(1):47–55.
[25] Constantz BR, Barr BM, Ison IC, Fulmer MT, Baker J, McKinney L, et al. Histological, chemical, and crystallographic analysis of four calcium phosphate cements in different rabbit osseous sites. J Biomed Mater Res 1998;43(4):451–61.
[26] Bohner M, Luginbuhl R, Reber C, Doebelin N, Baroud G, Conforto E. A physical approach to modify the hydraulic reactivity of alpha-tricalcium phosphate powder. Acta Biomater 2009;5(9):3524–35.
[27] Lu C, Miclau T, Hu D, Hansen E, Tsui K, Puttlitz C, et al. Cellular basis for age-related changes in fracture repair. J Orthop Res 2005;23(6):1300–7.
[28] Nikolaou VS, Efstathopoulos N, Kontakis G, Kanakaris NK, Giannoudis PV. The influence of osteoporosis in femoral fracture healing time. Injury 2009;40(6):663–8.
[29] Kyro A, Usenius JP, Aarnio M, Kunnamo I, Avikainen V. Are smokers a risk group for delayed healing of tibial shaft fractures? Ann Chir Gynaecol 1993;82(4):254–62.
[30] Passeri LA, Ellis Iii E, Sinn DP. Relationship of substance abuse to complications with mandibular fractures. J Oral Maxillofacial Surg 1993;51(1):22–5.
[31] Sloan A, Hussain I, Maqsood M, Eremin O, El-Sheemy M. The effects of smoking on fracture healing. Surgeon 2010;8(2):111–6.
[32] Frankenburg EP, Goldstein SA, Bauer TW, Harris SA, Poser RD. Biomechanical and histological evaluation of a calcium phosphate cement. J Bone Joint Surg Am 1998;80(8):1112–24.

[33] Lin CY, Kikuchi N, Hollister SJ. A novel method for biomaterial scaffold internal architecture design to match bone elastic properties with desired porosity. J Biomech 2004;37(5):623–36.
[34] Hollister SJ. Scaffold design and manufacturing: from concept to clinic. Adv Mater 2009;21(32–33):3330–42.
[35] Schneider P, Krucker T, Meyer E, Ulmann-Schuler A, Weber B, Stampanoni M, et al. Simultaneous 3D visualization and quantification of murine bone and bone vasculature using micro-computed tomography and vascular replica. Microscopy Res Tech 2009;72(9):690–701.
[36] Wehrli FW, Ladinsky GA, Jones C, Benito M, Magland J, Vasilic B, et al. In vivo magnetic resonance detects rapid remodeling changes in the topology of the trabecular bone network after menopause and the protective effect of estradiol. J Bone Mineral Res 2008;23(5):730–40.
[37] Kim K, Jeong CG, Hollister SJ. Non-invasive monitoring of tissue scaffold degradation using ultrasound elasticity imaging. Acta Biomater 2008;4(4):783–90.
[38] Ludwig W, King A, Reischig P, Herbig M, Lauridsen EM, Schmidt S, et al. New opportunities for 3D materials science of polycrystalline materials at the micrometre lengthscale by combined use of X-ray diffraction and X-ray imaging. Mater Sci Eng A 2009;524(1–2):69–76.
[39] Komlev VS, Mastrogiacomo M, Peyrin F, Cancedda R, Rustichelli F. X-ray synchrotron radiation pseudo-holotomography as a new imaging technique to investigate angio-and microvasculogenesis with no usage of contrast agents. Tissue Eng C: Methods 2009;15(3):425–30.
[40] Langer M, Liu Y, Tortelli F, Cloetens P, Cancedda R, Peyrin F. Regularized phase tomography enables study of mineralized and unmineralized tissue in porous bone scaffold. J Microscopy 2010;238(3):230–9.
[41] Komlev VS, Mastrogiacomo M, Pereira RC, Peyrin F, Rustichelli F, Cancedda R. Biodegradation of porous calcium phosphate scaffolds in an ectopic bone formation model studied by X-ray computed microtomography. Eur Cells Mater 2010;19:136–46.
[42] Willbold E, Witte F. Histology and research at the hard tissue-implant interface using Technovit 9100 New embedding technique. Acta Biomater 2010;6(11):4447–55.
[43] Milan JL, Planell JA, Lacroix D. Computational modelling of the mechanical environment of osteogenesis within a polylactic acid–calcium phosphate glass scaffold. Biomaterials 2009;30(25):4219–26.
[44] Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell 2009;5(1):17–26.
[45] Loosli Y, Luginbuehl R, Snedeker JG. Cytoskeleton reorganization of spreading cells on micro-patterned islands: a functional model. Phil Trans Royal Soc A: Math Phys Eng Sci 2010;368(1920):2629–52.
[46] Mogilner A, Wollman R, Marshall WF. Quantitative modeling in cell biology: what is it good for?. Dev Cell 2006;11(3):279–87.
[47] Olivares AL, Marsal E, Planell JA, Lacroix D. Finite element study of scaffold architecture design and culture conditions for tissue engineering. Biomaterials 2009;30(30):6142–9.