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EDITORIAL COMMENT

In Situ Heart Valve Tissue Engineering

Is Scaffold Structural Biomimicry Overrated?*

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We read with great interest the study by Uiterwijk et al. (1) in this issue of *JACC: Basic to Translational Science*, which represents one of the very few reports currently capable of providing comprehensive data on acellular synthetic heart valve scaffolds after 12 months of implantation in an ovine model. Uiterwijk et al. (1) fabricated implants with preferential fiber alignment (anisotropic tissue-engineered heart valve [aTEHV]) and with random arrangement (random TEHV [rTEHV]), with the hypothesis that the bioinspired anisotropic fiber architecture would facilitate the formation of native-like oriented collagen fibers in the circumferential direction. They reported that: 1) predefined fiber alignment in electrospun biodegradable heart valve scaffolds did not induce collagen organization; 2) although not statistically significant, a tendency for higher regurgitation and peak gradient was seen in the anisotropic TEHV; 3) the mechanics of all of the explants at 6 and 12 months were isotropic; and 4) substantial valve-to-valve variability was observed at the macroscopic and microscopic scales.

These unexpected findings seem to challenge the concept of biomimicry in tissue engineering and, similarly to other recent studies (2,3), pose an important and provocative question: if scaffold architecture is overruled by host tissue remodeling, what is the benefit of developing methods to prescribe a specific bioinspired architecture?

De novo collagen formation in vivo and in vitro is a multifactorial mechanism still poorly understood where, given the same form of contact guidance and external mechanical cues, different outcomes can be dictated by scaffold mechanical properties (4) or native extracellular matrix microstructure. Conversely, different hemodynamic loads and anatomic positions (arterial vs. venous, carotid vs. abdominal aorta) are associated with dramatically different fates of the implants (5). The role of the host's immunoresponse is pivotal, and mechanical stimuli on macrophages can have decisive consequences by inducing a specific phenotype.

A combination of the pore size and immuno- and mechanomodulated cellular responses could have resulted in limited macrophage infiltration, modest scaffold degradation, and extracellular matrix deposition. The tissue formation, as Uiterwijk et al. (1) themselves pointed out, was predominantly on the scaffold surface and not in its bulk, in between the fibers. In this way, the expected contact guidance by aligned polymer fibers did not come into play. This could have been further exacerbated by the loss of alignment as a consequence of fiber damage visible at 6 and 12 months, despite the fact that a considerable mass of polymer was still present at the end of the study. These considerations would re-enforce the notion that contact guidance is effective only under a number of other favorable circumstances.

Unexpected results were also represented by the mineralization seen in most of the valves at 6 months, which had not been detected in a previous study. Here, the different chemical composition of the material might have made the difference, but also, the different stiffness-dependent local loads in the scaffold could play a role, which, in turn, could have an effect on the osteogenic differentiation of smooth

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muscle cells. The dynamic deformation of the leaflets is complex and gives rise to nonuniform stress/strain distribution with the realistic conclusion that some of the results obtained on small samples might not have been representative of other regions of the valve.

Undoubtedly, Uiterwijk et al. (1) faced a rather difficult task in interpreting the results because of the high data variability, the limited number of animals (per group and time point), and the complexity of the topic. These aspects, together with the attempt to obtain as much information as possible by performing a wide range of analyses, inevitably restricted Uiterwijk et al. (1) to mainly speculate on their findings without the possibility of identifying a conclusive mechanistic explanation.

Although they rightfully recognized and discussed the study's limitations, we find that the indication of one of the scaffold architectures as random fiber distribution (rTEHV) misleadingly brings the reader to assume an isotropic construct, when, in reality, also the rTEHV featured a preferential fiber orientation and anisotropic mechanical properties. In this sense, this was a study on the comparison between 2 differently aligned scaffolds. A statistical analysis of the biaxial response before the implantation (Figures 1G and 1H in the article by Uiterwijk et al. [1]) was not presented, making the comparison of the 2 groups somehow indecisive. Also, the suggestion that the higher stiffness of rTEHV at 12 months might be due to collagen crosslinking is not well supported by experimental evidence and can be hardly correlated to differences in the fiber distribution in the explanted valves, because statistical analysis of the level of structural anisotropy was not provided.

Contrary to studies on heart valves, which require large-animal models, other relevant studies on the effect of hemodynamic load conditions (5) and scaffold fiber alignment (4) on endogenous remodeling of vascular grafts could take advantage of a higher number of animals by using a more financially sustainable and logistically convenient model like the rat. Although this allows for more accurate statistical evaluation of the experimental results, it still needs to be considered that macrophages from different species differ in their phagocytic activity, chemotactic responsiveness and sensitivity, and even size, thus questioning the relevance of the preclinical models and their capacity to recapitulate human immune responses (6). Therefore, the development of predictive in vitro test systems based on human cells and tissues, which go beyond the evaluation of viability, maturation, and activation of innate immune cells and include hemodynamic stimuli, might offer the possibility to study and unravel important immunodriven aspects such as scaffold degradation and tissue formation. In the same way, in silico models can significantly contribute to optimizing the design of the scaffolds, improving their preclinical outcomes, and ultimately enabling their translation to clinical settings. Computational models capturing the mechanics and mechanobiology of cardiovascular tissues as well as the immunodriven and mechanomediated growth and remodeling have already shown their capability to inform scaffold design and influence tissue engineering strategies. Further incorporation of the mechanosensitivity of macrophages and the interaction with other cells remains an ongoing effort.

As clinical and preclinical trials assessing implantable devices based on the notion of endogenous tissue restoration grow in number, predicting outcomes and identifying their associated device design variables is an urgent need to ensure safe and effective clinical translation.

Most importantly, the study by Uiterwijk et al. (1) remains unique in its capacity to document in vivo long-term effects of fiber alignment for the heart valve application. Their study re-enforces the importance of developing effective tools able to elucidate the complex mechanisms involved with long-term in situ remodeling. In this spirit, we agree with Uiterwijk et al. (1) that favorable results from in silico or in vitro models do not provide sufficient evidence in support of bioinspired approaches, and we recognize that efficacy of fiber alignment has never been demonstrated in a large animal model, and yet we think that the general question of whether or not prescribed scaffold structure is overruled by in vivo remodeling remains unanswered.

We believe that a constructive and collective debate is fundamental to advance the field of cardiovascular tissue engineering and to make the TEHV approach clinically relevant.

AUTHOR DISCLOSURES

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