Investigation of inflammatory response of decellularized porcine aortic tissue in mice: can we rely on this experimental setting?

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Keywords: Heart valve tissue engineering · Decellularization · Cellular reseeding · Xenograft rejection · Inflammatory immune response · Macrophages

With the article entitled ‘Alteration of inflammatory response by shock wave therapy leads to reduced calcification of decellularized aortic xenografts in mice’, Tepeköyulu et al. address the interesting as well as highly demanding topic of inflammation and calcification of xenogeneic bioprostheses for heart-valve replacement therapy, that both lead to early rejection of implants [1]. Commercially available...
(fixed) xenogeneic bioprosthetic valve devices, usually of porcine or bovine origin, are prone to rejection and subsequent degeneration in the post-implantation period. A possible solution in avoiding this fate could be indeed the use of tissue-engineered heart valve bioprostheses derived from allogeneic or even xenogeneic origin. Nonetheless, decellularized xenografts showed early degeneration and deterioration by mechanisms of inflammatory and immunological responses in human recipients during first clinical trials [2, 3]. In the study by Tepeköylü et al., the authors intend to introduce shock-wave therapy (SWT) as a novel tool to prolong xenogeneic bioprosthetic heart valve graft survival and in particular, to improve recellularization of xenograft valve. For this purpose, the authors describe the use of a specially adapted mouse model to analyse and compare in vivo reactions of a xenogeneic recipient’s immune system against subcutaneously implanted decellularized porcine aortic grafts with and without additional applied SWT. The authors examine, on one hand, explanted tissues after certain in vivo periods, with either histological, immunohistochemical or electron microscopic methods, and tested their contents for expressed proinflammatory cytokines using RT-PCR and, in addition, a certain cytokine profiler. On the other hand, they perform biomechanical tests on porcine decellularized aortic material and analyse the reseeding capacity of porcine decellularized aortic valves in a specially designed bioreactor system, again, with or without additional implementation of SWT, thus addressing the hypothesis that modulation of inflammation via SWT directly after graft implantation may positively influence graft survival by counteracting calcification.

Although the current study may seem very complex, reasonable and designed in a conclusive way at first glance, a more precise look reveals some limitations.

In general, it is difficult to extrapolate any insights from a mouse subcutaneous model to the human situation. Immune situations between mice and men are totally different (starting with expression of major antigenic epitopes like alpha-Gal), which can mislead the whole experimental setting. Macrophages are inflammatory cells and represent a crucial part of the host immune response. Certain macrophage populations may be beneficial, and, are in fact essential for rapid matrix turnover. Other macrophage subgroups are absolutely detrimental to graft survival and are part of prolonged inflammatory responses that finally leads to early graft failure (M1 vs M2 population). In the current manuscript, the authors consider this crucial point, but unfortunately they were unable to adequately address this point using the methods they applied. To our knowledge, CD163 is a common macrophage marker, that is not differentially expressed by Macrophage 1 and 2 populations. Moreover, there is absolutely no proof that C5/C5a and CD40L expression specifically promotes macrophage polarization towards proinflammatory M1 type, since CD40L, for instance, is mainly a marker for activated T lymphocytes. Additionally, TNF-α and interleukin 6 are only two out of many others inflammatory key players not analysed here and the real meaning of their increased release and a possible relationship to SWT is neither demonstrated nor proven by any method in the current study. Furthermore, certain patterns such as high-flow and pressure which may additionally affect heart valve tissues cannot be evaluated in a subcutaneous mode.

Mice are frequently used to model human disease states, to investigate pathological mechanisms or to test the efficiency of drugs. However, the direct translation of experimental data achieved in mice to human pathological events often fails due to significant differences of the immune system functionality between both species [4]. Novel mouse models such as immuno-deficient mice reconstituted with human haematopoietic cells may help to address some critical questions concerning human immunology, especially in xenograft transplantation.

Improvement in long-term durability of tissue-engineered grafts has been a subject of interest during the last decades. Novel insights typically emerge with basic research in the laboratory, and then evolve to clinical levels. Experiences gained from utilizing preclinical animal models display the relationship between basic sciences and clinical results. For more than 30 years, animal models have represented a crucial critical component of preclinical safety evaluation of prosthetic heart valves developed for human application. Prior to clinical trials, careful examination concerning biological responsiveness of novel potential (bio-)prosthetic material should be conducted in proper (!) animal experiments, since in vitro generated bioartificial tissues may behave entirely differently in a complex living host organism than previously expected.

In our opinion establishment of appropriate study protocols and careful selection of suitable animal models are crucial points in clarifying specific biological aspects and demonstrating the clinical safety of novel bioartificial implants.

**REFERENCES**


