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Letters

TO THE EDITOR

Evaluating Calcification in Tissue-Engineered Heart Valves

Much More Complicated Than Expected?

Van der Valk et al[1] assessed calcification in pulmonary tissue-engineered heart-valves (TEHVs) and reported occurrence in 35% of large animal implants mainly on a mild to microcalcific level. Further subgroup analyses showed no significant differences. They concluded that calcification did not significantly differ between tissue-engineered approaches or animal models but maintained that calcification represents a risk for TEHVs. Although the authors address a highly relevant topic, several results and conclusions should be taken with caution.

First, while calcification was the focus of this meta-analysis, the vast majority of the studies never intended calcification as key parameter and as such used adolescent or adult animals rather than weanlings. Schoen et al[2] clearly demonstrated the importance of using weanlings when aiming to assess calcification. As such, the nonsignificant age differences in studies using juvenile or adult animals for the observation of healing events is of limited relevance if juvenile is defined as beyond a weanling age of 14 weeks. Compared with the clinically pertinent calcification seen in weanlings (>80 μg/mg per leaflet) the 1 to 2 μg/mg seen in juvenile/adult animals highlights their unsuitability for calcification studies.[3] Hence, the multitude of subgroup analyses seems overstated. Yet, although only 35% of implants presented microspots to mild calcification, the value of this meta-analysis lies in creating awareness for the end goal of tissue engineering beyond functional living leaflets, which is the avoidance of calcific degeneration. While awareness for using "true" calcification models may follow in the wake, Van der Valk et al[1] also highlighted the need for standardized classification of tissue-engineered approaches.

By primarily discriminating between scaffold type and tissue-engineered approach, their classification is too imprecise to assess and compare remodeling outcomes. They classified xenografts under the same category as homografts and in vitro-grown tissue-engineered matrices (conditioned hydrogels), although the remodeling profile is substantially different given their heterogeneous nature.[4] While fresh decellularized homografts (NCT02035540) show excellent long-term performance, outcomes for xenografts remain unpredictable.[4] Tissue-engineered matrices harbor a strong intrinsic remodeling potential but display immature extracellular matrix organization at implantation. This cautions against grouping them altogether for a comparative outcome analysis. We therefore propose a less ambiguous classification in accordance with state-of-the-art nomenclature (**Figure 1A**).[4]

Second, the calcification scoring system applied appears equivocal, as it subsumes the authors' own analysis of published images and, in its absence, the description provided by the authors of the respective papers.[1] Supporting the concern of interpretation variance, the calcification example in Figure 2 in the paper may well qualify as microcalcification rather than mild calcification, as described in the original publication.

Third, the overall number of analyzed animals is low, which is further aggravated by the uneven distribution among groups further impeding subgroup analyses.

Collectively, the results should be seen as exploratory, rather than as confirmatory. More systematic data and standardized guidelines for TEHV studies (**Figure 1B**) are urgently needed.

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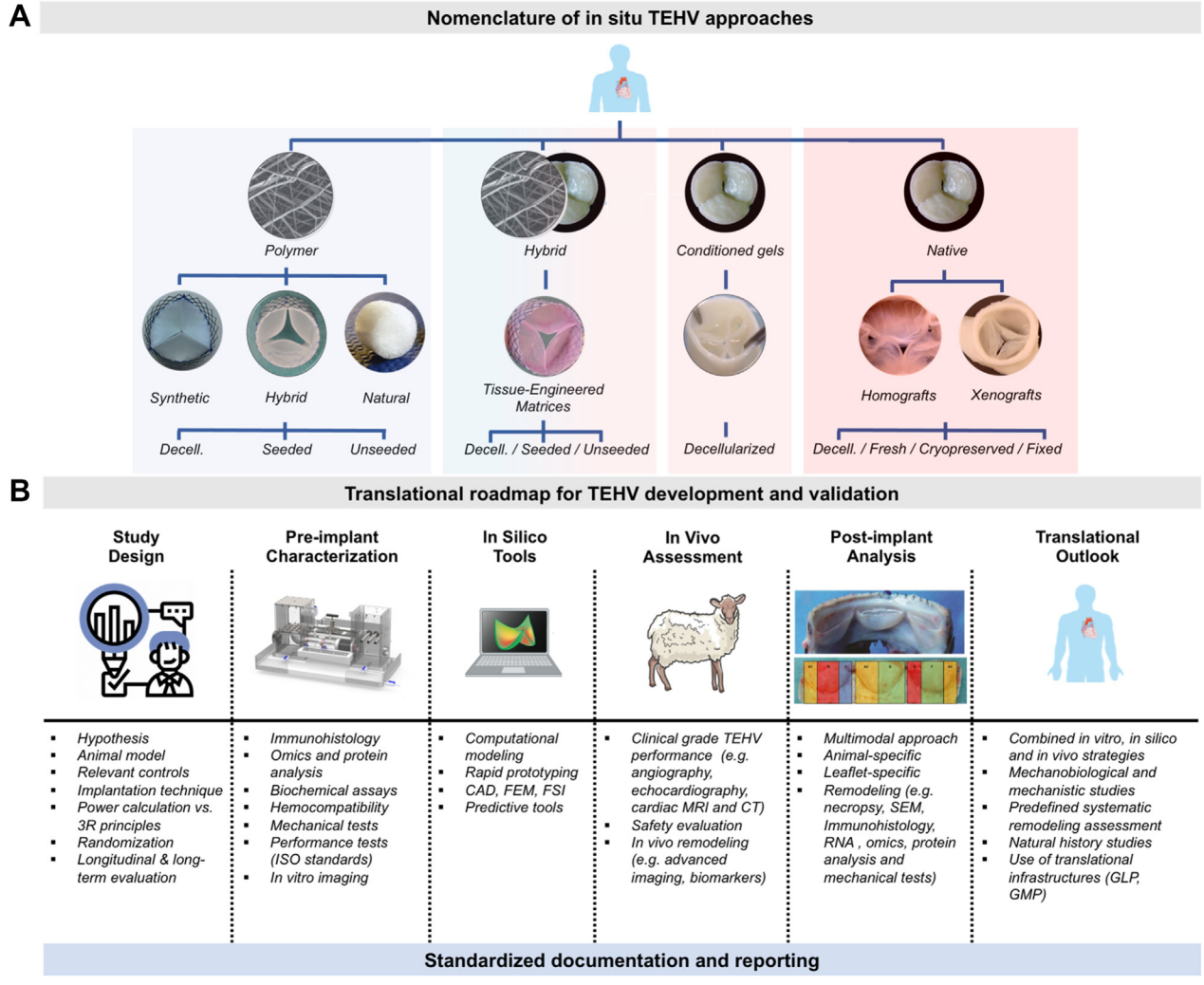
FIGURE 1 **Classification and Translational Roadmap for Tissue-Engineered Heart Valves**



(A) State-of-the-art nomenclature of in situ TEHV approaches and **(B)** translational roadmap for TEHV development. CAD = computer-aided design; CT = computed tomography; FEM = finite element method; FSI = fluid-structure interaction; GLP = Good Laboratory Practice; GMP = Good Manufacturing Practice; ISO = International Organization for Standardization; MRI = magnetic resonance imaging; SEM = scanning electron microscopy; TEHV = tissue-engineered heart valves.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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