Sex-dimorphic calcification dynamics in melt-electrowritten 3D engineered valve tissues modelling early calcific aortic valve disease

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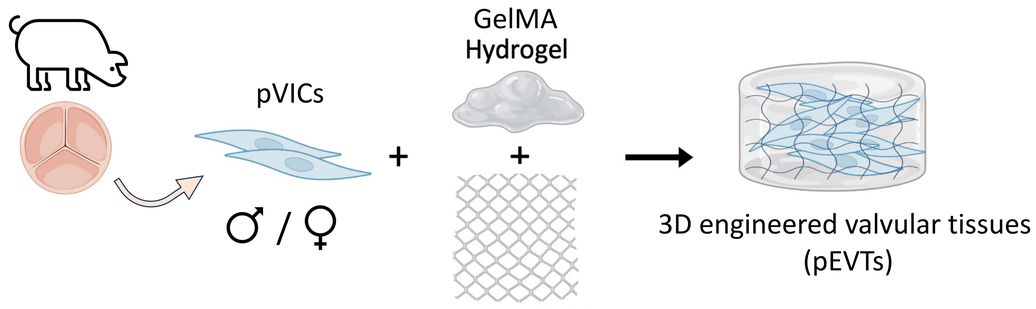
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INTRODUCTION

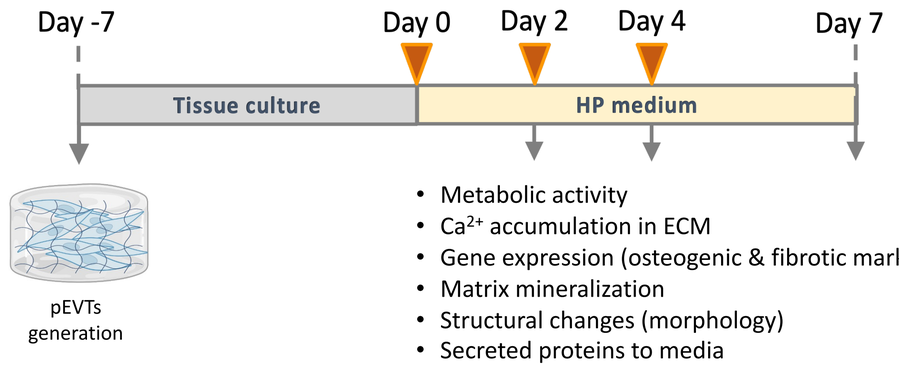
Calcific aortic valve disease (CAVD) is characterised by extracellular matrix (ECM) remodelling and progressive mineral deposition within the aortic valve. Clinical evidence reveals sex-dependent disease trajectories, where men develop a more calcific phenotype, while women exhibit a more pronounced fibrotic response during the disease progression. However, mechanistic investigation of these differences remains limited due to the lack of physiologically relevant in vitro models. The **AIM** of this project is to biofabricate a 3D valvular tissue model that enables the investigation of sex-specific responses to pro-calcific stimuli. Engineered valvular tissues (pEVTs) are generated by encapsulating primary valvular interstitial cells (pVICs) derived from male or female pig healthy donors within GelMA hydrogels reinforced with melt-electrowritten (MEW) PCL scaffolds. Calcification is induced through hyperphosphataemic (HP) stimulation to reproduce pathological remodelling. This platform allows the evaluation of osteogenic and fibrotic responses in a controlled 3D environment, with a specific focus on sex-dependent differences.

MATERIALS AND METHODOLOGY

Generation of 3D engineered valvular tissues with pVICs



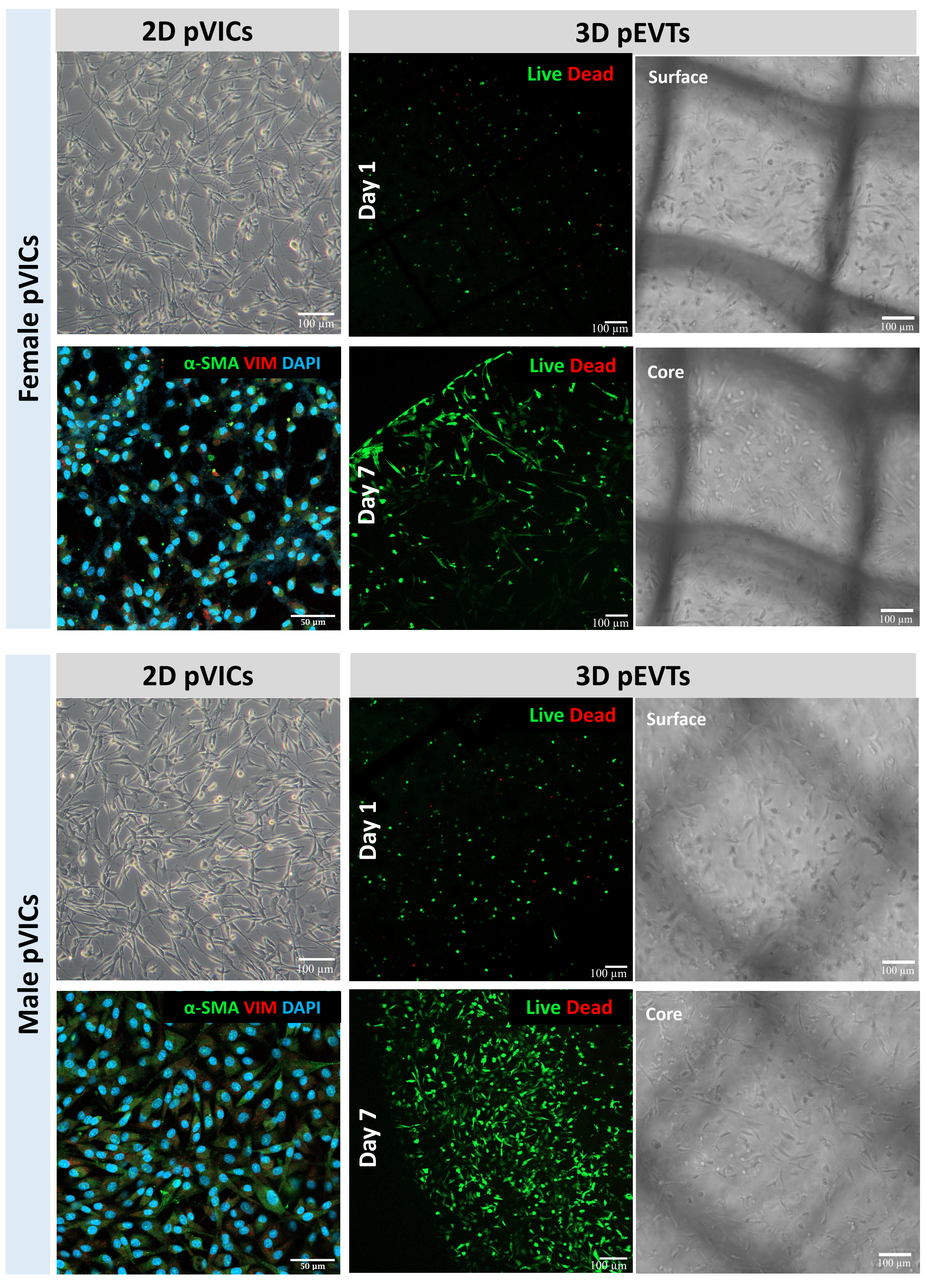
Calcific aortic valve disease induction by hyperphosphataemic stimulation



- Metabolic activity
- Ca^{2+} accumulation in ECM
- Gene expression (osteogenic & fibrotic markers)
- Matrix mineralization
- Structural changes (morphology)
- Secreted proteins to media

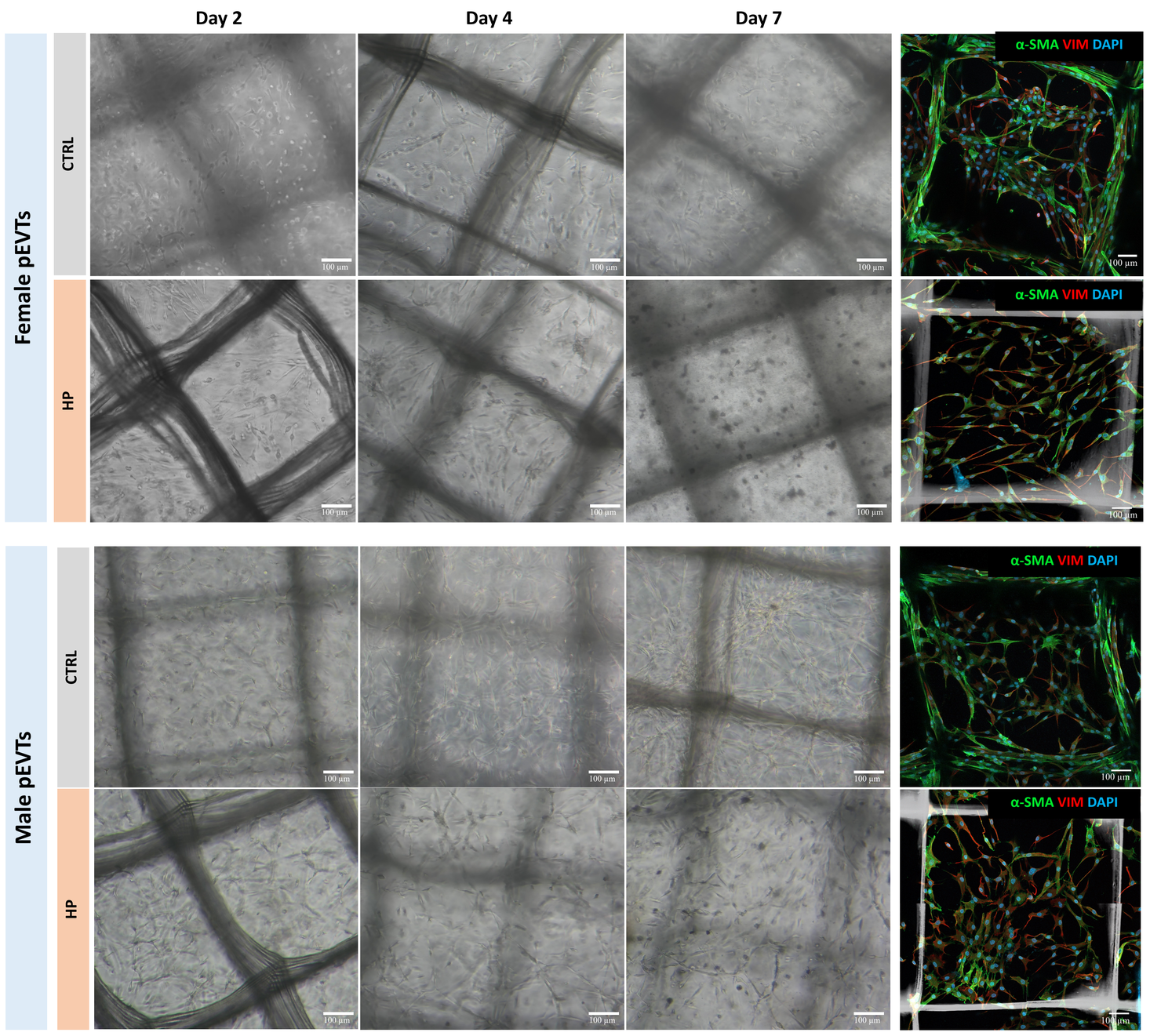
RESULTS

Sex-specific 3D valvular tissues characterization



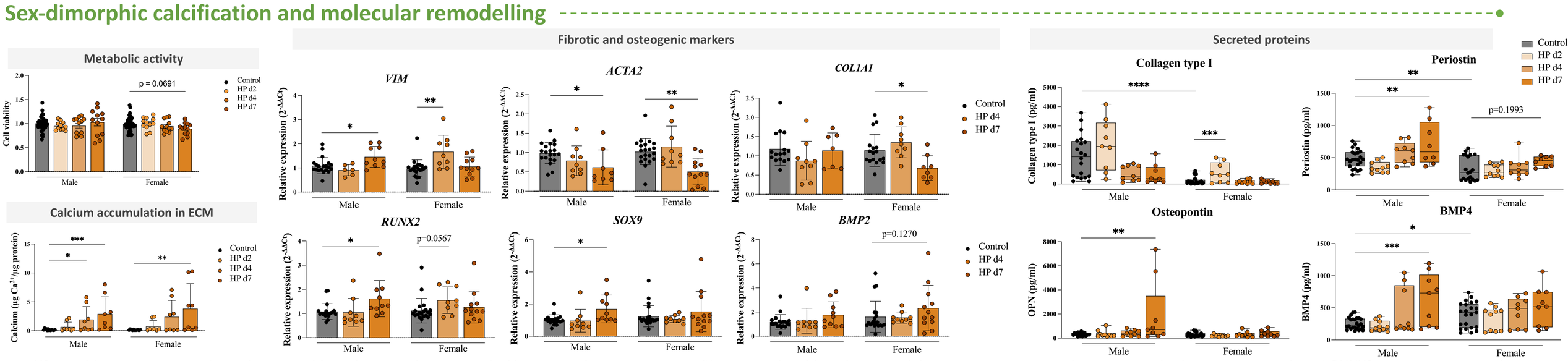
- pVICs were successfully isolated from male and female donors, displaying the typical spindle-shaped, fibroblast-like morphology. Female-derived cells appeared more elongated, whereas male cells showed a broader morphology.
- pEVTs displayed an homogeneous cell distribution across both surface and core regions. Live/Dead staining demonstrated high cell viability and progressive cell elongation from day 1 to day 7, indicating active matrix remodelling within the 3D environment.

Structural characterization under HP stimuli



- HP-treated pEVTs showed dark granular aggregates in both male- and female-derived constructs, consistent with early calcific deposits.
- Male-derived pVICs lost their elongated spindle-like morphology and adopted a more compact, polygonal appearance reminiscent of osteoblast-like phenotypes. In contrast, female-derived cells largely preserved their elongated morphology, with only modest reductions in α-SMA and limited cytoskeletal reorganisation, highlighting sex-specific remodelling responses.

Sex-dimorphic calcification and molecular remodelling



- HP conditions promoted rapid calcium deposition without major loss of viability, supporting early-stage calcific remodelling. Male tissues exhibited a stronger osteogenic response, with increased expression of RUNX2 and elevated secretion of osteogenic markers. In contrast, female tissues showed a transient and less sustained activation, consistent with a more fibrotic-like remodelling profile.

CONCLUSIONS

Biofabricated pEVTs recapitulate key features of early CAVD in a controlled 3D environment. The model reveals clear sex-dependent differences, with male tissues showing enhanced osteogenic commitment and female tissues displaying a more transient fibrotic response. Notably, hyperphosphataemic stimulation induces rapid calcific remodelling within a few days, comparable to conventional models requiring longer induction times. This platform supports the study of early disease mechanisms and opens the path towards fully human engineered valve models.

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