Introducing a novel ex-vivo calcification model for human aortic valves

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**Background:** Aortic stenosis (AS) is a narrowing of the aortic valve opening due to calcification and thickening of the leaflets. There are currently no effective pharmacological treatments for AS, and surgery or percutaneous interventions are the only therapeutic options. The cellular and molecular mechanisms underlying AS are complex and not fully understood due to the complexity and multi-factorial nature of AS and the absence of suitable test systems. In the current in-vitro models to study valvular calcification, isolated valvular interstitial cells (VICs) are cultured in 2D and in the absence of its native extracellular matrix. In addition, other cell types like valvular endothelial cells (VECs) and macrophages, which have been shown to impact the behavior of VICs, are absent.

**Purpose:** Our goal is to establish an ex-vivo calcification model for human aortic valves which maintains the original valve structure and cellular components.

**Methods:** Diseased aortic valves were harvested from patients undergoing aortic valve replacement. A 5mm x 7mm piece of this aortic valve tissue was dissected and embedded in agarose, then cut into 300 mm slices using a vibratome (Figure 1). In this way VICs remained in their valvular environment allowing the most native response of VICs to stimuli. The use of multiple slices from a single patient ensured proper internal controls. The slices were placed on a cell culture insert and cultured for 14 days. To induce calcification, the slices were cultured in the presence of 3 mM phosphate (n=23). As a control, valvular slices were cultured in the absence of 3mM phosphate (n=12). To validate if the calcification induced in the ex vivo culture system can be molecularly modulated the glucocorticoid dexamethasone was added to cultures containing 3mM phosphate (n=8). The slices were assessed for the presence of calcification using Alizarin Red staining and for the presence of osteogenic and inflammatory markers using (Immuno) histochemistry.

**Results:** In the presence of 3mM Phosphate, 74% of the slices demonstrated calcification, whereas no calcification was observed in all slices cultured without 3mM Phosphate (p<0.0001; Figure 2a). Furthermore, osteogenic genes such as Runx and alkaline phosphatase-1, as well as the inflammation marker NFκb were expressed in the calcified slices (Figure 2a). Dexamethason inhibited the calcification in the slices treated with 3 mM phosphate (Figure 2b).

**Conclusions:** We developed a novel ex-vivo calcification model for human aortic valves in which the initiation and progression of aortic valve calcification can be studied. In addition, the calcification can be modulated at a molecular level providing a unique opportunity to uncover the molecular signaling pathways involved in valvular calcification and to test compounds facilitating pre-clinical translational studies.
Figure 2