

NOTCH1-Targeted Endothelialized Patches for Bicuspid Aortic Valve Repair: Precision Strategies to Prevent Early Calcific Degeneration

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ABSTRACT:

This Perspective outlines a translational roadmap for NOTCH1-targeted endothelialized patches to prevent early calcific aortic valve disease (CAVD) in patients with bicuspid aortic valve (BAV). We synthesize NOTCH1 biology and single-cell insights that motivate spatial ligand presentation and mechanoresponsive patch design, contrast the proposed approach with current surgical materials, and define mechanical, biological, and regulatory performance targets. We propose prioritized preclinical experiments (in vitro NICD activation and VIC suppression assays; rabbit and porcine preclinical pathways), realistic timelines, and qualitative go/no-go criteria. Finally, we describe likely regulatory classifications and manufacturing quality-control metrics and outline three concrete first experiments to accelerate clinical translation. This Perspective is aimed at biomaterials engineers, translational cardiovascular scientists, and regulatory strategists pursuing disease-modifying valve repair.

KEYWORDS: *NOTCH1, bicuspid aortic valve (BAV), endothelialized patch, calcific aortic valve disease (CAVD), mechanotransduction, regenerative biomaterial*

1. Clinical Need and Translational Opportunity

The bicuspid aortic valve affects approximately 0.5–2% of the population and is the most common congenital cardiac valvular anomaly. BAV patients experience accelerated valve degeneration and earlier onset of CAVD than those with tricuspid valves. Contemporary surgical strategies include valve repair with autologous or xenogeneic pericardial patch augmentation, leaflet reconstruction, valve replacement (mechanical or bioprosthetic), and TAVR in selected cases. While repair can preserve native tissue, many currently used patch materials are biologically inert and do not actively modulate endothelial signaling implicated in VIC osteogenic transformation; several clinical series report structural valve deterioration and reoperation in the medium term (3).

Unmet needs succinctly stated: existing materials restore leaflet geometry but remain biologically inert and unable to correct flow- and genotype-driven disruption of endothelial NOTCH1 signaling. A repair patch that restores or sustains endothelial NOTCH1 activity at the blood-contacting surface could blunt upstream drivers of VIC osteogenic reprogramming and may reduce early calcification in at-risk patients with BAV (1,2).

Intended Clinical Niches:

- Primary BAV repair in younger patients who would benefit from native tissue preservation.
- Selective use in redo surgery when limited calcification is present, but leaflet geometry can be restored.

- Phenotype-guided indications (e.g., particular fusion phenotypes) to be refined with preclinical and early clinical evidence.

2. Rationale for Targeting NOTCH1

NOTCH1 is a transmembrane receptor central to endothelial homeostasis and valve development. In the aortic valve, NOTCH1 coordinates endothelial–interstitial cross-talk, modulates shear-responsive programs, supports eNOS signaling, and suppresses osteogenic transcription factors in VICs. Loss or dysregulation of NOTCH1 (through genetic variants or hemodynamic perturbation typical of BAV) leads to endothelial dysfunction, diminished NO signaling, inflammatory priming, and VIC osteogenic transition. This body of evidence supports NOTCH1 as a mechanistically relevant target for a biologically active patch (1,2).

Design Implications:

- NOTCH1 acts within a network that includes the BMP, Wnt, and TGF- β pathways; targeting NOTCH1 can blunt a proximal driver even when disease is multifactorial (1).
- Single-cell and spatial transcriptomic studies show spatial heterogeneity of NOTCH1 expression across valve leaflets, motivating spatial ligand presentation rather than homogeneous coating (1).
- NOTCH activation is ligand-dependent (Jagged/DLL family) and mechanically modulated; combining ligand presentation with microtopography that restores physiologic shear is therefore synergistic (1,2).

3. How the Proposed Patch Differs from Current Materials

The proposed patch differs from standard pericardial materials and existing endothelialized biomaterials in three main ways:

- Spatially patterned NOTCH ligand presentation informed by single-cell data (1).

- Combined mechano- and ligand-based activation (microarchitecture to normalize shear plus ligand cues) (1,2).
- Integrated potency QC (NICD induction assays as a lot release/potency metric) rather than relying solely on sterility and mechanical testing (1,2).

At the same time, well-established technologies (NO-releasing chemistries, fiber alignment, micropatterning) serve as enabling platforms integrated with the aforementioned novel design elements. Reviews of tissue-engineered heart valves and biomaterials approaches provide helpful context for incorporating these elements (4,5).

4. Materials and Fabrication Strategy

Luminal layer options: pre-seeded autologous endothelial cells, allogeneic “off-the-shelf” endothelial monolayers, or acellular surfaces functionalized with immobilized NOTCH ligands (Jagged/DLL mimetics). Each approach balances regulatory complexity, immunogenic risk, and signaling durability (1,2).

Microarchitecture: oriented microfibers and micropatterned grooves promote endothelial alignment and physiologic shear sensing; mechanical compliance should approximate native leaflet flexural stiffness to avoid aberrant mechano-transduction (4,5). Ligand presentation: covalent tethering or affinity-mediated immobilization of NOTCH ligands with controlled density and spatial gradients; co-presentation of eNOS-stabilizing elements (or NO-releasing chemistries) can reinforce shear–NOTCH coupling (1,2).

Manufacturability: validate ligand density and bioactivity (NICD induction), sterility, endotoxin, and storage stability (6,7).

5. Performance Targets

Mechanical

- Flexural rigidity within $\pm 20\%$ of native leaflets.
- Fatigue durability target: $\sim 10^8$ bending cycles.

- Suture retention tensile strength is adequate for surgical handling.

Biological

- NICD nuclear localization in endothelial cells within 24–72 h in vitro.
- ≥30% reduction in VIC RUNX2 expression vs control in co-culture osteogenic challenge.
- Restoration of shear-aligned endothelial morphology and eNOS activity comparable to healthy valve endothelium.
- Hemocompatibility per ISO 10993-4 thresholds (platelet adhesion, thrombin generation) (8,9).

6. In-vitro and Preclinical Roadmap (realistic timelines and models)

In-vitro (3–6 months): NICD immunostaining/qPCR, flow-chamber shear alignment and eNOS assays, VIC osteogenic co-culture under cyclic strain, hemocompatibility, and mechanical testing (1,2).

Small-animal (6–9 months): rat subdermal biocompatibility and rabbit aortic/patch models for endothelial retention and early calcification assessment. Endpoints: endothelial coverage, inflammation, microcalcification (Alizarin Red/histology) (5).

Large-animal (12–24 months): porcine/ovine leaflet augmentation with altered shear or ex vivo flow loops; endpoints: echocardiographic gradients, micro-CT/histologic calcification scoring, endothelial integrity, thrombosis, and immune response. Note: spontaneous BAV large-animal models are limited; surgical/flow modifications are pragmatic alternatives (4,5).

Illustrative sample sizes: small-animal n = 6–12/group; large-animal pilot n = 4–8/group with formal power calculations for pivotal studies.

7. Failure modes and mitigation

NOTCH1 overactivation (theoretical remodeling/hyperplasia), thrombogenicity of ligand coatings, immune responses to non-autologous cells/ligands, endothelial denudation under high shear, loss of ligand activity, and patch delamination. Mitigation: conservative ligand dosing, hemocompatibility testing per ISO 10993-4, accelerated aging and retention assays for ligands, ex vivo high-shear testing, and iterative mechanical validation (8,9).

Table 1: Design Lever → Target → Assays

Design Lever	Target	Assays/Methods
Surface ligand density	NOTCH1 activation	NICD staining, qPCR
Microtopography	Endothelial alignment, NO production	Flow chamber, eNOS assays
Compliance	Physiologic bending mechanics	Flexural testing
Ligand stability	Sustained signaling	ELISA, release kinetics
Hemocompatibility	Low thrombogenicity	Platelet adhesion tests

8. Regulatory considerations

Regulatory expectations: likely a Class III device in the U.S.; if living cells or bioactive ligands are present, the product may be classified as a combination product (device + biologic), which increases regulatory and manufacturing expectations. Early regulatory interaction (pre-IDE / pre-submission) is recommended to agree on classification, GLP safety studies, potency assays, and early clinical endpoints. Guidance on combination products and CGMP for combination products should be consulted during development (6,7).

CMC and QC: validated assays for ligand density and potency (NICD induction), sterility, endotoxin, storage stability, and validated sterilization compatible with ligand activity (6–8).

Clinical trial design: an early feasibility single-arm study focusing on safety and surrogate valve-function endpoints (transvalvular gradients, effective orifice area, and freedom from device-related complications at 1 year) is a reasonable first step before randomized pivotal trials (6).

Table 2: Preclinical Roadmap

Stage	Duration	Endpoints	Illustrative n
In vitro	1–3 mo.	NOTCH1 activation, VIC suppression	—
Small animal	1–3 mo.	Inflammation, early calcification	6–10
Large animal (short-term)	3–6 mo.	Hemodynamics, signaling	4–6
Large animal (long-term)	6–12 mo.	Calcification, durability	6–8

9. Limitations, equity, and access

Manufacturing complexity and cost may limit availability in low-resource settings. A lower-complexity acellular ligand scaffold may offer a pragmatic, lower-cost pathway that reduces regulatory complexity and broadens access (5,6).

10. Conclusion - immediate next steps

- Establish an in-vitro potency assay: standardized endothelial NICD induction assay (immunostaining + qPCR) and a VIC osteogenic suppression assay; set numeric go/no-go thresholds (e.g., $\geq 30\%$ RUNX2 suppression) (1,2).

- Run a pilot small-animal study comparing standard pericardial patch vs NOTCH1-ligand patch in a rabbit aortic/patch model focused on endothelial retention and early calcification (6-month readout) (3,5).
- Schedule a pre-IDE/pre-submission with the relevant regulator to confirm product classification, GLP expectations, and acceptable early clinical endpoints (6,7).

Conflict of Interest

None declared.

Ethical Statement

Not applicable.

AI Use Statement

Generative AI tools were used only for phrasing and grammar; all scientific concepts and conclusions are original.

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Abbreviations

BAV - bicuspid aortic valve;

CAVD - calcific aortic valve disease;

VIC - valvular interstitial cell;

eNOS - endothelial nitric oxide synthase;

NICD - NOTCH intracellular domain;

NO - nitric oxide;

PMA - premarket approval (US FDA).

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