3D alginate scaffold for anatomical aortic valve tissue engineering

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Abstract: *Within the field of biomedicine, alginate applications are numerous, from wound healing and cell transplantation to delivery of bioactive molecules. Recently, alginate based biomaterials are entering into clinical trials for the treatment of myocardial infarction (1). Due to its non-thrombogenic nature, this polymer is very promising for cardiac applications, including scaffold for heart valve tissue engineering. One essential property of alginates in this respect is the possibility to form virtually any shapes (films, fibers, beads) in a variety of sizes. Alginate solutions can form gels under mild conditions in the presence of calcium, by displacement of sodium ions and resulting attraction of the alginate molecules. Our aim is, therefore, to fabricate three-dimensional (3D) alginate scaffolds mimicking precisely the anatomical shape of human aortic valves, as a substrate for valve tissue engineering and repair.*

Background

The aortic heart valve (AHV, see **Figure 1**) is responsible for the unidirectional blood flow between the left ventricle and the aorta. It is a highly demanding environment, where the valve opens over 100,000 times each day is subjected to shear stress, bending (1), strain and loading forces. While most humans will have normal function of the valve, some will need AHV replacement surgery, due to several problems, such as aortic insufficiency, stenosis and aortic aneurysm. It is estimated that the annual number of patients requiring HV replacement in 2050 will be 850,000 worldwide (2).

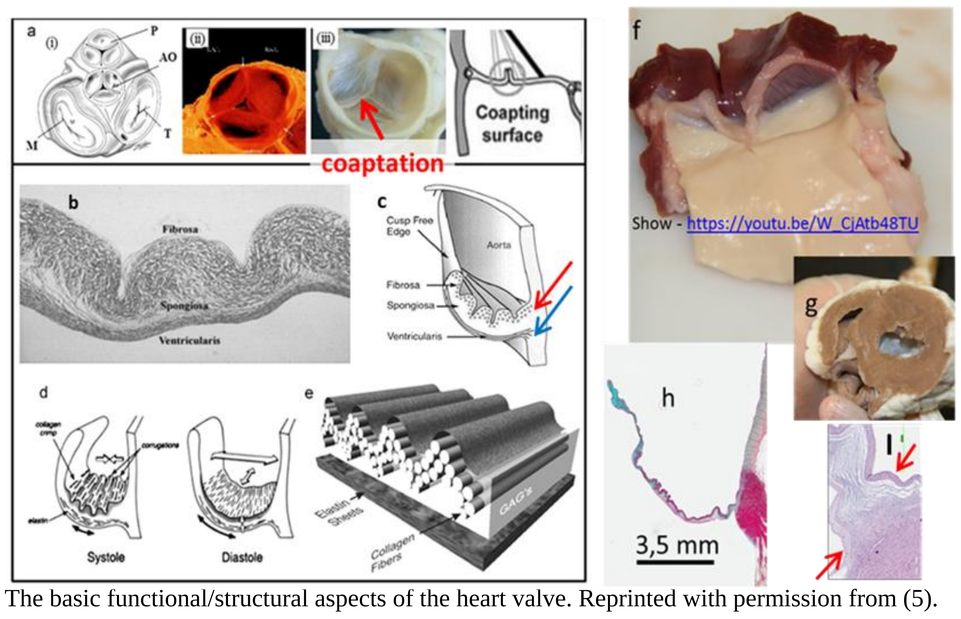

Figure 1. The basic functional/structural aspects of the heart valve. Reprinted with permission from (5).

Commonly, the valve is replaced with a mechanical or bioprosthetic valve. The problems with these substitutes are, for mechanical valves, the patient will need to commit to a lifetime of anticoagulants, and bioprosthetic valves are limited by tissue degradation. An alternative that has been widely studied is the use of a **biodegradable scaffold** that has a mechanical integrity close to the aortic valve, where cells can grow and remodel the structure and that the final product will be a functional autologous aortic valve. The scaffold must be able to mimic the complexity of the AHV and its performance resulting from natural communication processes occurring in the tissues, allowing the valve to work properly (3, 4).
Thus, to achieve this complexity, the substitutes must host cells that will be able to be expressed and produce an extracellular matrix that will function in a similar way to the native valve (1, 5-8).

Methods

To prepare scaffolds that can reproduce the complex geometry of aortic heart valves, we made use of the gelling properties of alginate solutions. Briefly, the geometrical and structural design of a typical aortic heart valve (1, 2, 8) was obtained using Blender software ((9), see **Figure 2** – bottom panel). The generated 3D file was converted into the stereolithography (STL) format and 3D printing performed in Objet Eden260VS – 3D printer (Stratasys, Edina, Minnesota, USA) using light-curable polyacrylate monomers.

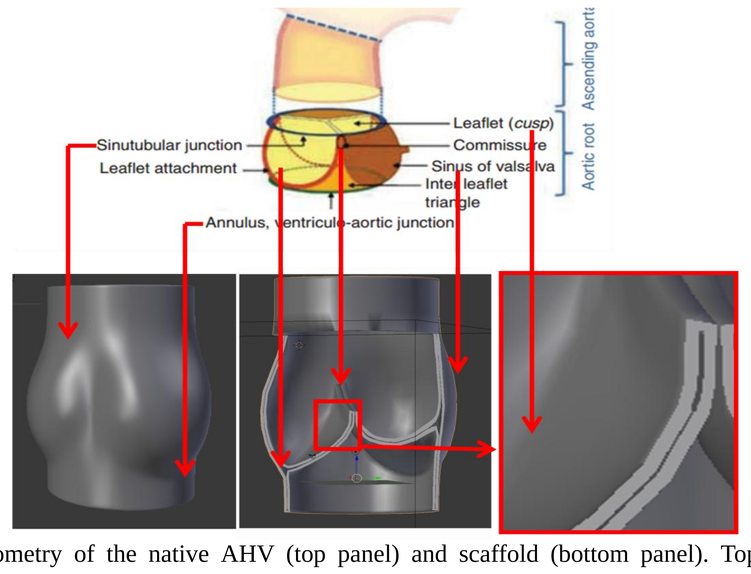

Figure 2. The geometry of the native AHV (top panel) and scaffold (bottom panel). Top panel was reprinted with permission from (1).

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After printing the supporting material was removed which yielded a flexible valve-like structure with sinuses of Valsalva and tree coapting leaflets. Subsequently, agarose moulds were obtained by casting agarose saturated in $CaCl_2$ solution (2% w/w) into the 3D printed form. Finally, alginate scaffold preparation was carried out by immersing the $CaCl_2$-saturated agarose moulds into alginate solutions (see **Figure 3**).

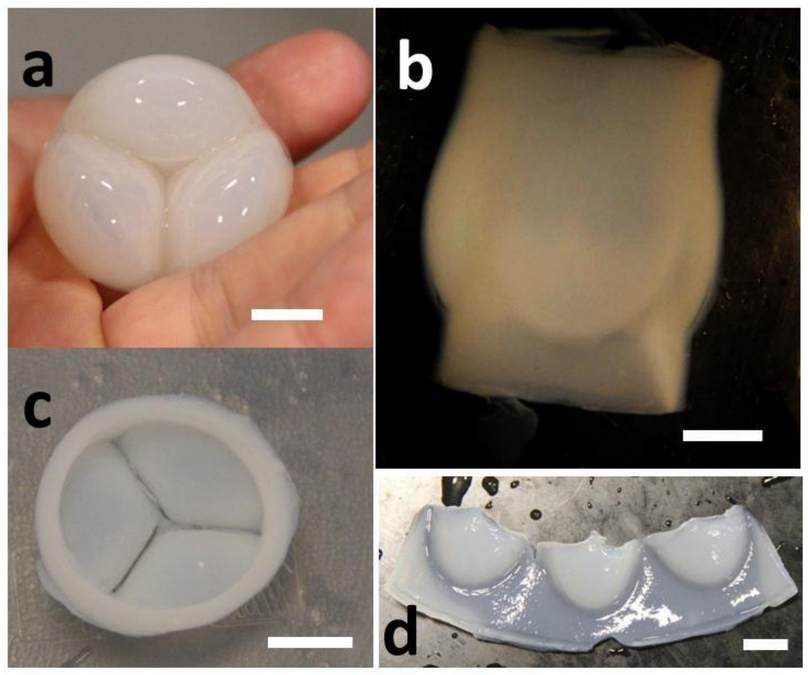

Figure 3. Alginate shaped in the tricuspid valve; ventricular view (a), side view (b), hinge - atrial view (C), and open valve view (D). Scale bars are 1 cm.

Results

Calcium ions diffused from the agarose mould effectively cross-linked alginate solution in close vicinity, leading to a creation of an alginate gel layer. **The increase of $CaCl_2$ concentration in agarose caused an increase in materials constancy and its transparency** due to the crosslinking process.
The agarose mould could be easily removed in a subsequent step. The resulting alginate structure closely matched the agarose mould geometry and hence the 3D printed replica of a human aortic valve. Moreover, by extending the length of mould immersion into sodium alginate solutions, scaffold thickness and composition could be controlled. Such control allowed forecasting further improvement to facilitate cellularisation and tissue formation, and to improve mechanical properties (see **Figure 3**).

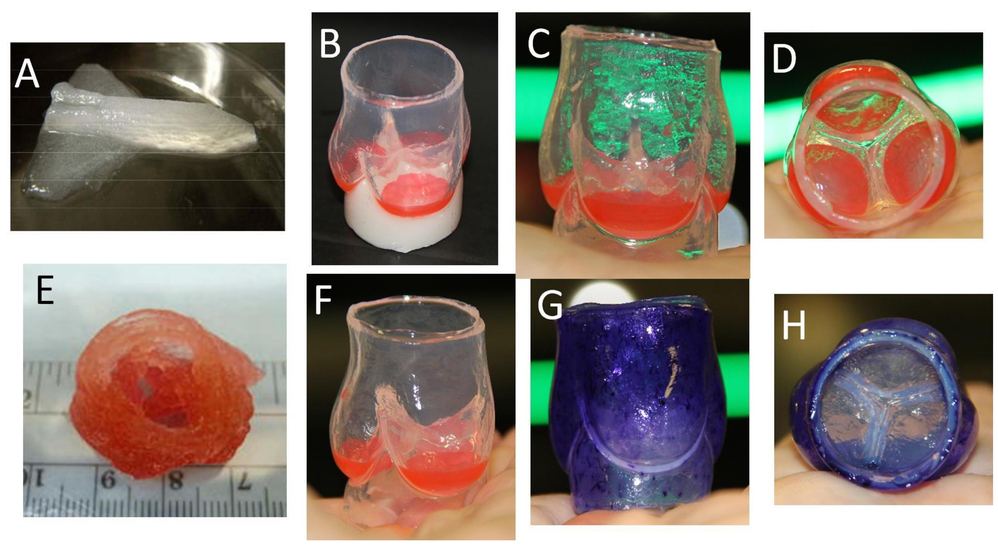

Figure 4. Various possible hydrogels formations obtained from alginate. (A) Alginate aircraft were printed with highest, previously reported resolution, using reactive extrusion method. Reprinted with permission from (5); multicomponent alginate valve on the post (B) and after post removal (C), a view from the ventricular site (D). HV printed with highest, previously reported precision (E), versus HV, obtained with 3D printing assisted method (F). Blue Valsalva (G) and transparent leaflets combined in one construct. (E) was reprinted with permission from (10).

Conclusion

Alginate can form versatile and tunable hydrogels which can be cast in 3D configurations that mimic the shape of a human aortic valve (see **Figure 4**). As preparation steps can be freely adjusted to incorporate viable cells, such structures could serve as a basis for *in vitro* tissue formation, which would further improve mechanical properties of the hydrogel. Also, the ease of chemical modification and functionalization of alginate with cell ligands provides rational tools to increase cell interactions and attract cells *in situ*, which are significant steps in the formation of functional valves *in vivo*.
Overall, this novel and flexible technique that can be readily integrated with other strategies present an extraordinary potential to create the "ideal" scaffold for producing a living valve substitute.

Bibliography:

1. Chester AH, El-Hamamsy I, Butcher JT, Latif N, Bertazzo S, Yacoub MH. The living aortic valve: From molecules to function. Glob Cardiol Sci Pract. 2014;2014(1):52–77.
2. Yacoub MH, Takkenberg JJM. Will heart valve tissue engineering change the world? Nat Clin Pract Cardiovasc Med. 2005;2(2):60–1.
3. Dohmen PM, Konertz W. Tissue-engineered heart valve scaffolds. Ann Thorac Cardiovasc Surg Off J Assoc Thorac Cardiovasc Surg Asia. 2009 Dec;15(6):362–7.
4. Dohmen PM. Tissue engineered aortic valve. HSR Proc Intensive Care Cardiovasc Anesth. 2012;4(2):89–93.
5. Hasan A, Ragaert K, Swieszkowski W, Selimović S, Paul A, Camci-Unal G, et al. Biomechanical properties of native and tissue engineered heart valve constructs. J Biomech. 2014 Jun 27;47(9):1949–63.
6. Yacoub MH, Kilner PJ, Birks EJ, Misfeld M. The aortic outflow and root: a tale of dynamism and crosstalk. Ann Thorac Surg. 1999 Sep;68(3 Suppl):S37–43.
7. Arjunon S, Rathan S, Jo H, Yoganathan AP. Aortic valve: mechanical environment and mechanobiology. Ann Biomed Eng. 2013 Jul;41(7):1331–46
8. Yacoub MH. In Search of Living Valve Substitutes*. J Am Coll Cardiol. 2015 Aug 25;66(8):889–91.
9. Introduction — Blender Reference Manual [Internet]. [cited 2015 Nov 23]. Available from: http://www.blender.org/manual/getting_started/about_blender/introduction.html
10. Duan B, Hockaday LA, Kang KH, Butcher JT. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. J Biomed Mater Res A. 2013 May;101(5):1255–64.