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Development of a three-dimensional printed heart from computed tomography images of a plastinated specimen for learning anatomy

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Abstract: Learning anatomy is commonly facilitated by use of cadavers, plastic models and more recently three-dimensional printed (3DP) anatomical models as they allow students to physically touch and hold the body segments. However, most existing models are limited to surface features of the specimen, with little opportunity to manipulate the structures. There is much interest in developing better 3DP models suitable for anatomy education. This study aims to determine the feasibility of developing a multi-material 3DP heart model, and to evaluate students' perceptions of the model. Semi-automated segmentation was performed on computed tomgoraphy plastinated heart images to develop its 3D digital heart model. Material jetting was used as part of the 3D printing process so that various colors and textures could be assigned to the individual segments of the model. Morphometric analysis was conducted to quantify the differences between the printed model and the plastinated heart. Medical students' opinions were sought using a 5-point Likert scale. The 3DP full heart was anatomically accurate, pliable and compressible to touch. The major vessels of the heart were color-coded for easy recognition. Morphometric analysis of the printed model was comparable with the plastinated heart. Students were positive about the quality of the model and the majority of them reported that the model was useful for their learning and that they would recommend their use for anatomical education. The successful feasibility study and students' positive views suggest that the development of multi-material 3DP models is promising for medical education.

Key words: Three-dimensional printing, Anatomical education, Educational tools, Multi-material and-color, Segmentation

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Introduction

In most undergraduate medical schools, anatomy is traditionally taught using human cadavers. Cadavers provide good representation of true anatomy, thus increasing the likelihood of retaining knowledge [1]. Plastination techniques have revolutionized the preservation of the human body or organs. Plastinated cadavers are treated with silicone-like materials to have a long shelf life so that they can be reused in classrooms. Nonetheless, it is very expensive to maintain plastination facilities, procure full inventory of the specimens, or replace them if they are damaged, which may happen more frequently when there is increased handling in large student populations [2]. This may place a financial burden on teaching institutions [3]. Hence, there is an interest in identifying new methods of reproducing the existing plastinated specimens. One promising way to do this is to replicate them using three-

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dimensional printed (3DP) anatomical models. These models can be created with 3D printing, an additive manufacturing (AM) process that deploys a 3D printer to print materials layer by layer to form a physical object. Fig. 1 shows the basic steps on how the 3DP models can be created.

Ideally, 3DP models should provide three-dimensional visualization with high spatial resolution and anatomically correct details, and allow tactile feedback to help better memorize anatomical structures. Such features are critical for students [3-9]. Recent studies have shown that 3DP models can be advantageous for teaching anatomy in medical schools with no access to cadavers, whether due to the lack of financial resources or cultural and ethical considerations [10, 11]. Other applications of 3DP models include surgical planning, patient-specific implant design, tissue engineering and forensic medicine [3-9].

Despite numerous benefits of 3DP models, several authors have highlighted that more can be done to enhance the learning process, such as adding multiple colors and textures to the models [12]. Colors may help students recognize and memorize important anatomical structures more easily [12]. Materials of different textures may also allow a user to experience different sensations when touching and feeling different soft tissues, therefore may help develop tactile skills in their learning of anatomy [10, 13]. While this avenue has yet to be fully explored, research work on multi-colored and multi-material 3DP models is starting to gain momentum as a result of better access to AM machines and technological advancements in 3D printing materials and colors [14-16]. Fused deposition modelling (FDM), vat photopolymerization, powder-binder jetting, sheet lamination and material jetting are some of the AM technologies used to 3D print various parts of the human body [5].

It is important to determine what the printed models are going to be used for. This goes hand in hand with the capabilities of the AM technology because the type of material, deposition and curing process for each technology is different, resulting in printed models with very different attributes, such as strengths, compliance, weight, color, cost, speed of manufacturing and surface finish [5, 8]. For example, in the printing of heart anomalies for surgical planning and implant design, powder binder jetting and FDM is the preferred choice due to faster printing times and lower running costs [5, 17]. Powder binder jetting uses powder-based materials such as plaster and starch and FDM uses thermoplastic materials such as nylon and polycarbonate. Unfortunately, powder prints tend to be highly porous and require further post-processing of chemicals to increase their strength and durability [18], and thermoplastic ones are rigid when produced [5]. These reduce their attractiveness for the production of durable and pliable anatomical models for teaching and learning [12].

Material jetting, while not commonly used due to its high

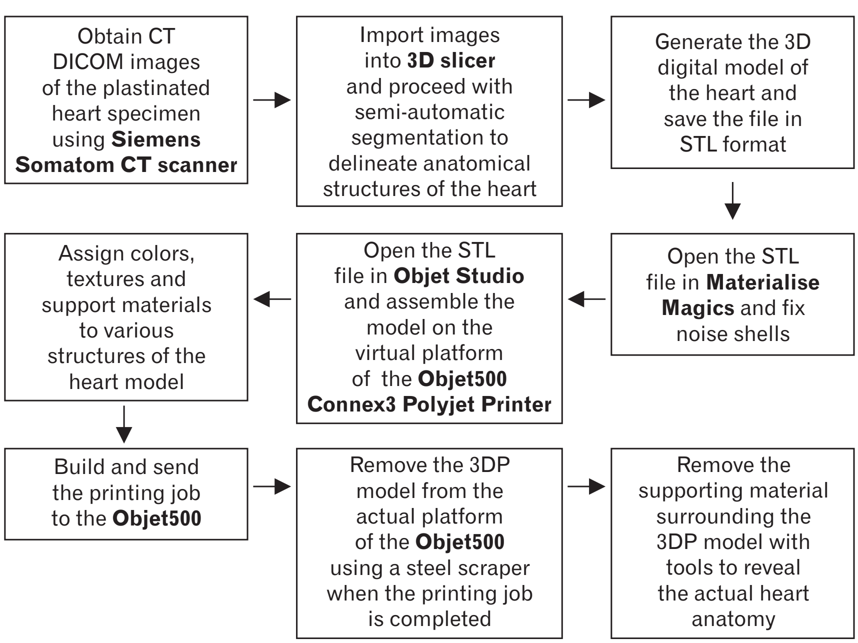

Fig. 1. Workflow process on three-dimensional printing (3DP) of the heart using computed tomography (CT) images of its plastinated specimen. DICOM, Data Imaging and Communications in Medicine; STL, stereolithography.

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printer maintenance and material costs [19], appears to be superior over other AM technologies for medical education. This is because it can print models with high accuracy and flexibility, and provides excellent surface finish that closely mimics various soft and hard tissues in the human body. It is also able to produce strong and compliant models with long lifespans, and can be re-used in the demanding environment of teaching labs and classrooms [12, 20]. The term compliant refers to structures that are able to bend and deform when touched. This technology ejects liquid photopolymers from the printing head, cures the liquid with laser and deposits material layer by layer on the build platform. Other authors have supported the use of material jetting because a combination of polymers can be selected to produce varying textures and colors [21], and transparent materials can be chosen to visualize specific morphology relative to neighboring or deep organs in a single printing job [22]. Subsequently, this also suggests that material jetting is more time-efficient compared to FDM since the latter is unable to print multiple materials simultaneously [13].

To date, literature pertaining to multi-color, multi-material 3DP anatomical models developed specifically for the purpose of medical education is limited [12]. One study looked into the development of neurosurgical models for surgical training and created multi-layers of varying soft tissue textures such as the brain tumor and dura mater [20]. The study suggested that different textures can be created to replicate actual anatomy with material jetting technology. Bartel et al. (2018) [22] also recognized that given the flexibility of the material, it is possible to use these models to assess the positioning of implants or devices on patient-specific heart anatomy. Other studies used computed tomography (CT) images of patients to develop 3DP models but were only targeting specific regions of the heart such as such as valves and ventricles, and were used for informing patients rather than for teaching anatomy [23-27]. Although they are good for patients' education and surgical planning, they are not ideal for medical education due to insufficient anatomical details.

At present, the group is not aware of any studies that use CT images of plasinated specimens to reconstruct a whole 3DP heart by material jetting. Therefore, for the first time, this study reports the use of a plastinated specimen to develop a multi-material 3DP heart suitable for learning anatomy. We also assessed morphometric differences between the printed model and the plastinated heart and students' views on these models.

Materials and Methods

Computed tomography scan data acquisition

One plastinated heart specimen (Gubener Plastinate GmbH, Guben, Germany) belonging to the Lee Kong Chian School of Medicine (LKCMedicine), Nanyang Technological University (NTU), Singapore was scanned using a 64-slice Somatom Definition Flash CT scanner (Siemens Healthcare, Erlangen, Germany) with 1-mm slice thickness and 50% overlap to obtain Data Imaging and Communications in Medicine (DICOM) images. CT data of patients or cadavers was not used in the study due to ethical constraints and no access to cadaveric materials. These images were imported as a stack into the open-source imaging software 3D slicer version 4.8.1 [28] for creating the 3D digital anatomical models.

Segmentation process

In 3D slicer, structures of the heart such as the ventricles, atria, coronary arteries, large vessels, pulmonary and aortic valves were initially identified and delineated by applying semi-automated segmentation to create their 3D digital surfaces. This process involved selecting appropriate greyscale threshold values corresponding to the region of interest in each image slice [29, 30]. After completing the segmentation process (Fig. 2A) and generating the 3D digital model of all the heart structures, they were each saved as a stereolithography (STL) file format (Fig. 2B) which is recognizable by the 3D printer (Objet 500 Connex3 Polyjet Printer, Stratasys Ltd., Eden Prairie, MN, USA), and then imported into 'Materialise Magics' version 20 (Materialise NV, Leuven, Belgium). 'Magics' is an STL editor software used for post-processing of 3D files so as to prepare them for 3D printing.

Creating three dimensional printed models

Fixing STL errors

In Magics, individual 3D structures of the heart are commonly referred to as 'shells' and the surface of each shell is connected by hundreds of small triangles. These shells often contain noise as a result of the segmentation process. They consist of floating, intersecting, overlapping and misconnected triangles that should be removed from the model. These need to be fixed in order to ensure that the files are printable and can produce good surface finish. This was done by removing, realigning, and patching the problematic triangles through Boolean operators and applying global shrink wrap-

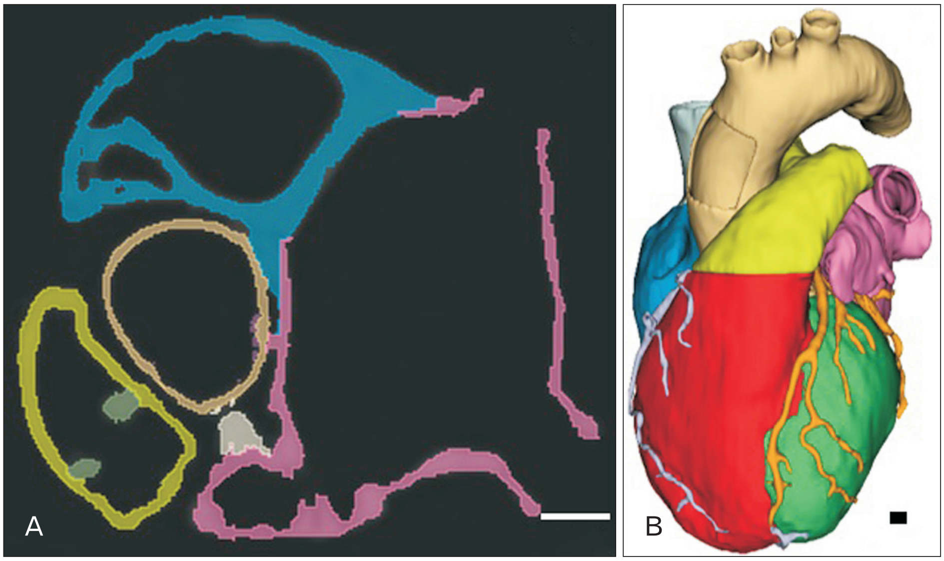

Fig. 2. The three-dimensional digital heart model after completion of the segmentation process. Arbitrary colors were assigned to individual structures for easy recognition during segmentation (A) and saved as stereolithography format (B). Scale bars=1 cm (A, B).

ping to remove errors inside the model.

Printing the model

After all the shells have been fixed, they were assembled together as a model in 'Objet Studio,' a 3D printing software that supports the Objet 500 Connex3 Polyjet Printer. This printer uses material jetting technology, has the capability to allow users to assign different colors and textures to each shell, and is available at the Singapore Centre for 3D Printing (SC3DP), NTU, Singapore. In Objet Studio, each shell is assigned in either blue or pink shades using a combination of rigid opaque photopolymers (Stratasys Ltd.) and translucent elastomers (Stratasys Ltd.) to create various soft and stiff textures. The cardiac valves, superior vena cava and pulmonary trunk were selected to be of softer and more flexible material while the chambers and arteries were more rigid. In areas of the digital model where there are voids, overhangs or delicate structures such as the inner walls of the ventricles, aorta and valves, water-soluble support materials (Stratasys Ltd.) were added as reinforcement to prevent the structures from collapsing when it is being printed and cured layer by layer. Finally, the model was sent for 3D printing on the 3D Polyjet printer, the Objet 500.

When the printing process is completed, the 3DP model will entail both the support material and the actual printed anatomy. Therefore, the support material needs to be removed to reveal and isolate the anatomical print of the heart. This was done manually by peeling the material by hand, water-jetting, and using thin metal spatulas.

Fig. 1 shows the complete workflow of creating the 3DP heart. Overall, the entire process took approximately 2.5 weeks to complete.

Morphometric analysis

Measurements of both the 3DP model and plastinated heart were taken at various anatomical locations using a vernier caliper (Kern, Germany). This was to determine the extent of errors produced in developing the 3DP model. These measurements consisted of diameters of the aorta, pulmonary trunk, superior vena cava, circumflex artery, left anterior descending artery, right coronary artery, and posterior interventricular artery. Length and breadth of the heart were also measured. Specific points for each of the above measurements are defined in Table 1. Then, the weight of the 3DP model and plastinated heart were measured using an electronic weighing scale (model WTB 2000, Radwag Wagi Elektroniczne, Radom, Poland). All measurements were done three times and the means were calculated.

Evaluation of the models

Anatomy at LKCMedicine is taught during first 2 years of a 5-year MBBS program according to a systems-based integrated curriculum. Plastinated human specimens are important teaching tools for gross anatomy instruction. Four copies of the 3DP model were used as a teaching resource along with plastinated heart specimens during the cardiorespiratory teaching block for year 1 undergraduate medical students during academic year (AY) 2018–2019 (n=138). As a follow up study, students were

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Table 1. Description of measurements for each anatomical structure

Parameter	Place of measurement
Heart	Length: From the apex to the mid-point of the superior margin of the base of the heart Breadth: At the broadest part of the heart in the transverse direction
Aorta diameter	At the origin of the left subclavian artery
Pulmonary trunk diameter	At bifurcation
Superior vena cava diameter	At the point of entry to the right atrium
CX diameter	Near origin Near termination
LAD diameter	Proximal part Middle part Distal part
RCA diameter	At the conus branch At the inferior margin Just before PIA Proximal PIA

CX, circumflex artery; LAD, left anterior descending artery; RCA, right coronary artery; PIA, posterior interventricular artery.

invited to provide their views on the 3DP model in AY 2019–2020. They were asked to answer four survey items based on a Likert scale (1=strongly disagree to 5=strongly agree). These items focused on colors, features of the 3DP model and its usefulness for learning anatomy. Two open-ended items were also included for students to comment on what they liked about the 3DP model and suggestions on how to improve its quality. All these items were made accessible to the students through Qualtrics (Qualtrics LLC., Provo, UT, USA).

Data analysis

To determine the morphologic differences between the plastinated heart and 3DP model, the percentage error was derived by taking the absolute of (mean of plastinated specimen minus 3DP model) divided by plastinated specimen, times 100 [31]. A two tailed paired *t*-test was done in Microsoft Excel 2016 (Microsoft, Richmond, WA, USA) to determine if measurements between the 3DP model and plastinated specimen were statistically significant ($P<0.05$). The survey data were presented as the mean, standard deviation (±SD) and range of scores. The Cronbach alpha was also calculated to determine internal consistency of the survey items.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (NTU's Institutional Review Board Reference No. IRB-2016-06-020 and IRB-2014-11-015) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was not applicable as the specimen was procured from Gubener Plastinate GmBH (Guben, Germany) that preserves donated bodies by plastination. This article also does not contain patient data.

Results

3DP heart

The 3DP model created from segmenting CT scans of a plastinated heart specimen was found to contain a wealth of anatomical details, including the delicate, thin branches of the right and left coronary arteries (Fig. 3). This suggests that the segmentation process was effective and reliable. The pulmonary trunk and the superior vena cava were assigned in blue (Fig. 3A, B), the valves in translucence (Fig. 3C), and the rest of the heart in pink (Fig. 3A, B). Additionally, the 3DP model was found to be pliable and compressible (Fig. 4).

The measurements of the 3DP heart model compared to the plastinated heart specimen are shown in Table 2. Ten out of 15 measurements showed no significant differences between the plastinated heart and 3DP model ($P>0.05$). The left and right coronary artery diameters contained a wider range of errors (2.75%–27.68%) compared to diameters of other anatomical structures (0.22%–4.84%).

The total material consumption and costs (excluding printer cost and manpower) for printing the 3DP heart were ~1,228.5 g and ~SGD (Singapore dollars) $411.20 respectively (Table 3). The weight of the 3DP heart model and the plastinated heart were 232.63 g and 155.97 g, respectively.

Students' opinions

Fifty-eight students out of 137 students (42.33%, aged 19–23) responded to the survey. The Cronbach alpha value was recorded as 0.77 revealing satisfactory internal consistency of the survey. Overall, students' survey evaluations were very positive and implied that the 3DP heart model was a useful resource for their learning. Table 4 displayed the Likert scale survey results. Majority of the students (88%) either agreed or strongly agreed that 3DP model helped with their learning while 83% of the students felt that color coding assisted them in recognizing the features on the model. Most of the students

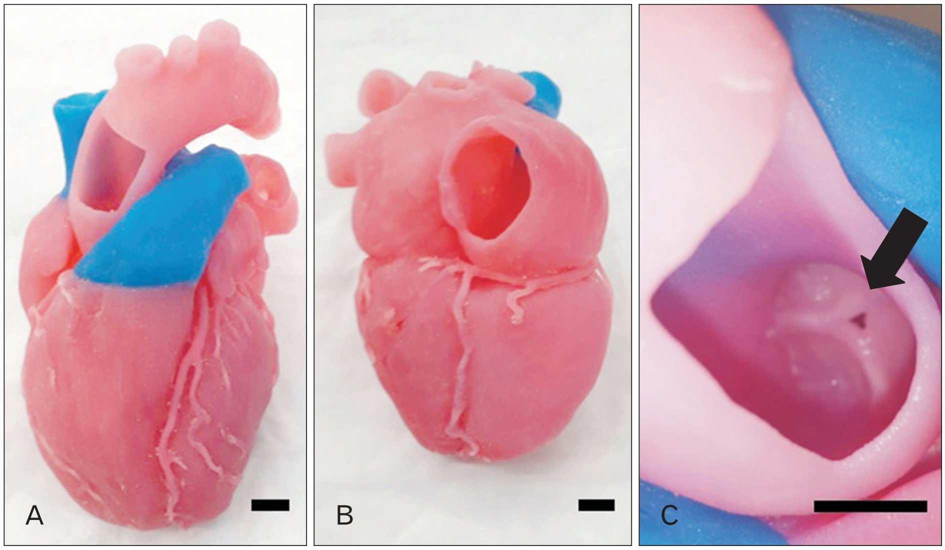

Fig. 3. The completed three-dimensional printed full heart model. (A, B) The anterior and posterior views of the printed heart respectively. (C) The aortic valve (arrow). Scale bars=1 cm (A–C).

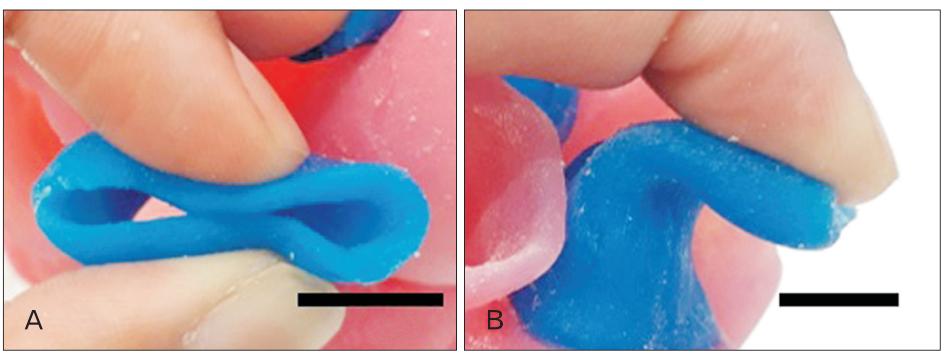

Fig. 4. The superior vena cava showing structural compressibility (A) and pliability (B). Scale bars=1 cm (A, B).

Table 2. Mean measurements, percentage errors and *P*-values comparing the plastinated and three-dimensional printed heart

Measurement	Plastinated heart	3DP heart	Error (%)	*P*-value
Heart				
Length (mm)	111.50	113.33	1.64	0.09
Breadth (mm)	76.83	76.67	0.22	0.74
Weight (g)	155.97	232.63	49.16	0.001
Aorta diameter (mm)	18.33	18.60	1.45	0.75
Pulmonary trunk diameter (mm)	18.00	17.67	1.85	0.67
Superior vena cava diameter (mm)	15.83	16.60	4.84	0.22
CX diameter (mm)				
Near origin	3.63	3.53	2.75	0.23
Near termination	2.77	2.62	5.42	0.04
LAD diameter (mm)				
Proximal part	3.33	2.60	22.00	0.002
Middle part	2.49	2.22	10.98	0.003
Distal part	1.80	1.97	9.26	0.30
RCA diameter (mm)				
At the conus branch	3.47	3.30	4.81	0.13
At the inferior margin	2.95	3.77	27.68	0.002
Just before PIA	2.77	2.63	4.82	0.18
Proximal PIA	1.53	1.77	15.22	0.07

CX, circumflex artery; LAD, left anterior descending artery; RCA, right coronary artery; PIA, posterior interventricular artery.

Table 3. Material consumption and costs for printing one three-dimensional printed full heart

Type of material	Consumption (g)	Cost (SGD $)
VeroCyan	17.3	8.50
VeroMagenta	133.0	65.80
TangoPlus	267.3	154.40
Support	811.0	182.50
Total	1,228.5	411.20

SGD, Singapore dollars.

Table 4. Survey items and students' responses concerning the three-dimensional printed heart

Survey item	Mean±SD	Range of scores (1–5)
1. The 3D printed heart model is anatomically accurate.	4.24±0.63	2–5
2. The 3D printed heart helped me learn anatomy.	4.36±0.69	3–5
3. The colors on the 3D printed heart helped me recognize anatomical structures.	4.16±0.79	2–5
4. I would recommend the 3D printed modes as a teaching tool for learning anatomy.	4.27±0.64	3–5

Likert scale points: 1, strongly disagree; 2, disagree; 3, neutral; 4, agree; 5, strongly agree (number of participants=58).

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felt models are accurate (93%) and recommend as a teaching tool (90%). Free text comments of the students revealed that 3DP model is easy to maneuver and handle with no stress or fear of damage. Majority of the students emphasized that color coding helped better visualization and identification of different structures of the heart.

"*Less fear when handling, no stress*"

"*It's much clearer to see the different structures of the heart as compared to the plastinated specimen*"

"*Different colors that allow us to identify different structures and the anatomical accuracy*".

Comments on improving the 3DP model were largely on using more/better colors, textures and details, making it more durable, making removable structures, and having more variations of heart anomalies:

"*not sure if it is possible, but would be interesting to have models of hearts with pathologies studied in the course such as VSD/tetralogy of fallot*"

"*allow more views of the heart by having reflectable or removable parts*"

"*The colors were too bright and stark and not accurate (bright yellow and pink)*"

Overall, majority of the students implied that the 3DP model was useful as a learning tool.

Discussion

In this study, the use of material jetting technology to produce a 3DP heart and students' evaluations were reported. The 3DP model was created by acquiring CT data from a plastinated heart specimen, performing semi-automated segmentation on the CT images to create a 3D digital model, fixing the surface and assigning materials and colors to the model, and 3D printing and cleaning the model. Regardless of 3D printing technologies, many researchers have used patient CT data to print heart models, however limited to regions of interest such as the aorta, ventricles, valves or arteries [23-27, 32-34]. This prototype is an initial, preliminary version of a full 3DP heart produced by material jetting and based on CT scans of its plastinated specimen for future deployment in medical education.

Benefits with regard to the development of the 3DP heart are numerous. With different elasticities attributed to individual anatomical structures, a user is able to feel different textures for different parts of the heart. This allows distinguishing between softer and stiffer materials of individual structures. To a certain extent, the flexibility and compressibility of the model may also allow students to move the structures and enable them to look deeper structures, subsequently helping to improve tactile perception [12]. The application of a multi-material 3DP heart can be further extended to the clinic as it could help a patient differentiate between normal and abnormal structures using different tactile properties. In addition, some students suggested having pathological versions of the 3DP model. Examples include the calcification of valves or ventricles [35]. Since the model is compressible and flexible unlike FDM or powder-based prints which are rigid, this could encourage a physically interactive learning environment for users [36, 37].

Color-coding offers instant visual recognition and clarity of separate heart structures compared to the plastinated specimen, as reported in this study. This is particularly useful for students learning the fundamentals of anatomy [12, 13]. On the other hand, students suggested that it would be better if the colors of the heart ventricles were less bright, and more anatomical details can be added. The possibility of incorporating the suggested enhancement to the colors is constrained by the limited choices available in the market. Moreover, anatomical details of the 3DP model matched the plastinated specimen. To have more structural details, CT data of patients rather than plastinated specimens could be used to create a new 3DP model. In addition to the 3DP model, its corresponding digital 3D model (generated during the segmentation process) could become a learning resource for students. Digital 3D model enhances further interaction by rotating, zooming and panning in the coronal, sagittal, or axial planes. Additionally, locating and hiding of the structures can be achieved in the software to design learning exercises or assessment [38, 39].

It was found that printing materials for the 3DP heart was far cheaper (~SGD $411.20) than the plastinated heart specimen (~SGD $6,000) which was routinely used for anatomy teaching at our medical school. This suggests that it may be more cost effective to print models in the long run, in agreement with McMenamin et al. (2014) [3]. Material jetting printing materials (~SGD $2,000) and printer (~SGD $400,000) are currently expensive compared to FDM (~SGD $100–$8,000) or powder-binder jetting material (~SGD $0.55/cm^3) and printer (~SGD $65,000). However, prices may come down in the future as material jetting technology advances. The digital model may also be used to print as many replicates as needed using any 3DP technology. Based on this, medical schools can look forward to using 3DP models as a practical and cost-saving solution in order to overcome financial or cultural barriers.

Certain dimensional differences were detected between the 3DP model and plastinated heart. Possible reasons for this observation is the accumulation of segmentation errors in several parts of the workflow process to produce the 3DP model. When performing segmentation using CT images of the plastinated specimen, air pockets were seen in some parts of the muscle, and this makes the identification of tissue boundaries more difficult. In particular, the valves were very thin and the branches of coronary arteries not easy to locate so this required more time to segment manually. It may be possible that the plastination process or when storing the specimen over extended periods of time could have affected the quality of the CT images. Moreover, choosing a smaller or larger threshold value, filtering, smoothing and expanding the structures were done to ensure that the pockets were filled, resulting in segmentation inaccuracies. These most likely explained why the range of errors largely varied, especially in the coronary arteries of the 3DP heart model (2.75%–27.68%). Other sources of errors may include the file conversion process from DICOM to STL [40]. In spite of these errors, the morphological measurements were in a similar range to other reported studies [41-43]. Furthermore, since the purpose of the study was to determine if it was feasible for use as a teaching tool in classrooms, these errors would not have changed the outcome of the study.

The mean weight of the 3DP heart was considerably more (232.63 g) than the plastinated heart (155.97 g), resulting in the highest percentage error of 49.16%. Nonetheless, the weight of the 3DP model was within the normal range of a real cadaveric heart (170–510 g) [44]. This is likely due to residual support material left in the ventricles of the 3DP model. Lighter weight of the plastinated heart may owe to dehydration and chemical treatment during plastination.

Although the focus of the study was to test the feasibility of printing an anatomically accurate 3DP heart model, this could be further extended to patients where such models may be created in patient-specific cases and used to help doctors explain the difference between normal and anomalous heart conditions by assigning different material properties to such structures. Examples of anomalies include the calcification of valves or ventricles [35]. It can also help surgeons foresee challenges that they may encounter during surgery [22, 45]. Future work may include investigating accurate segmentation methods and material properties of the prints so as to develop models with dimensional and tactile accuracy, which could then be very useful in surgical planning, simulation and anatomy learning. Suggested solutions for accurate segmentation include the acquisition of higher quality CT images using fresh frozen cadaver specimens [46], actual patient data [25, 27] or angiograms to ensure that all internal structures of the heart are intact and the delineation of soft tissue boundaries is clearer and more precise. For developing models that closely represent the feel of actual anatomy, bio- and synthetic polymers like gelatin and polyurethane may be considered, and mechanical compositions of photopolymers like viscoelasticity, elastic modulus and shore hardness to mimic soft tissue and organs may be examined [47]. Reliability of the proposed method may be examined with a larger sample size. As reported by the students, investigating the feasibility of printing other complex anatomies may also be carried out, which will be useful for them to learn various heart conditions in classrooms.

The segmentation of the mitral and tricuspid valves was excluded from the study as their quality in CT scans were insufficient for segmentation. Nevertheless, a proof of concept was carried out with a prosected half of another plastinated heart specimen, with the mitral and tricuspid valves intact. Initial results show that it was possible to 3D print these internal structures (Fig. 5). More studies are needed to verify its quality assurance.

In conclusion, printing a multi-material 3DP model of the heart was possible using material jetting. It is anticipated that it can become practical and cost-effective teaching tool for

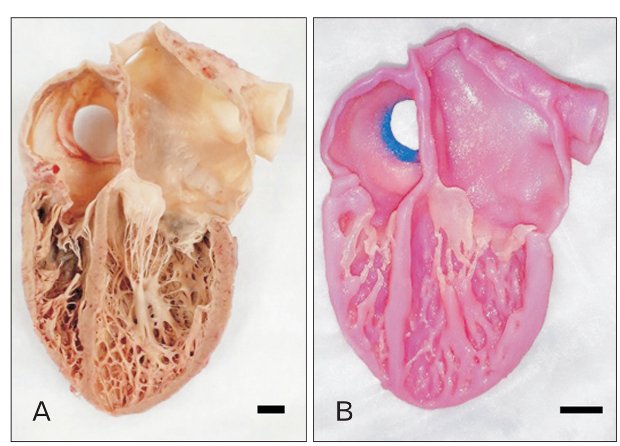

Fig. 5. (A, B) Three-dimensional printing of the prosected half of a plastinated heart specimen showing mitral and tricuspid valves. Scale bars=1 cm (A, B).

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medical schools to support anatomy teaching. Pliability and color coding of the structures offer sensory feedback to users which may facilitate the process of learning anatomy. As the plastinated specimen was obtained from a real human cadaver, the 3DP model bears all the individuality of the original living heart. Preparing a range of 3DP models from several plastinated specimens will reinforce the sense of the subtle variability seen between organs from different donors.

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Conceptualization: SR, SRM. Data acquisition: SR, HKJT, GJST, SRM. Data analysis or interpretation: SR, HKJT, MAF, SRM. Drafting of the manuscript: SR, HKJT, GJST, WYY, MAF, MLB, SRM. Critical revision of the manuscript: SR, WYY, MAF, NLB, SRM. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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