Artificial Organs

Detection of Thrombosis in the Extracorporeal Membrane Oxygenation Circuit by Infrasound: Proof of Concept

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Abstract: As of today, there exist no reliable, objective methods for early detection of thrombi in the extracorporeal membrane oxygenators (ECMO) system. Within the ECMO system, thrombi are not always fixed to a certain component or location in the circuit. Thus, clot fragments of different shapes and consistencies may circulate and give rise to vibrations and sound generation. By bedside sound measurements and additional laboratory experiments (although not detailed herein), we found that the presence of particles (clots or aggregates and fragments of clots) can be detected by analyzing the strength of infra-sound (< 20 Hz) modes of the spectrum near the inlet and outlet of the centrifugal pump in the ECMO circuit. For the few patients that were considered in this study, no clear false positive or negative examples were found when comparing the spectral approach with clinical observations. A laboratory setup provided insight to the flow in and out of the pump, confirming that in the presence of particles a low-amplitude low-frequency signal is strongly amplified, enabling the identification of a clot. **Key Words:** Clot detection—Centrifugal pump—Extracorporeal membrane oxygenation.

Initially, extracorporeal membrane oxygenators (ECMO) gained use in the treatment of infants with pulmonary and/or cardiac failure. Given recent advances, there is now enough evidence to support a widespread use of ECMO also in adults. For instance, Peek et al. (1) considered a large number of patients with acute respiratory distress syndrome induced by the H1N1-virus, which were treated with ECMO. The success of this treatment clearly demonstrated the potential of the extracorporeal system.

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Despite the above-mentioned success, the ECMO system is associated with increased levels of hemolysis and thrombogenesis. These seemingly counteracting processes may occur simultaneously although being interdependent. Moreover, there is a dependency on the local flow environment with both processes often found in turbulent flows, in flows with high shear-rates as well as in stagnation regions of the flow (cf. Kameneva et al. [2]). These flow features occur to different extent depending on the type of pump used in the ECMO system. However, both hemolysis as well as thrombogenesis have clinically been observed in different types of pumps, such as centrifugal and roller pumps. For example, Chiu et al. (3) considered thrombus formation in a left ventricular assist device (LVAD) whereas Barrett et al. (4) compared different types of pumps for the ECMO system. The main finding was that ECMO systems using centrifugal pumps are associated with an increased risk of hemolysis that in turn is likely to contribute to other organ injuries. However, in a later paper, Barrett et al. (5) reported that in spite of the fact that centrifugal pumps are more likely to induce ECMO-related complications, there is no difference in survival rate between the groups using roller as compared to centrifugal pumps. The authors point out that there is a need for further research to optimize the use of centrifugal pumps and form strategies to prevent ECMO-related complications.

Currently, the detection procedure is based on clinical judgment of laboratory data and optical examination of the tubing system. An algorithm for pump thrombus management was proposed by Goldstein et al. (6). However, as clearly pointed out by the authors, this therapeutic algorithm is based on experience and there was a lack of objective data to properly assess the algorithm. Thus, the need for a noninvasive and objective method to detect thrombi in blood pumps still remains.

The idea of using acoustic data to aid clinical decision-making is by no means new. Such means have been proposed for detecting end-of-life of LVAD (7), and detecting thrombosis in implant rotary blood pumps (8,9). Carrying out an in vivo study, Kaufmann et al. (9) considered the sound signature of the HeartWare (Framingham, MA, USA) HVAD LVAD System. A quantitative approach for acoustical detection of pump thrombosis was proposed. The approach was based on spectral analysis of the acoustic signal of the ventricular assist device. The authors considered 105 patients among which 8 had signs of pump thrombosis that later was confirmed, that is, after pump replacement. With pump thrombosis, an increase in the strength of the pump

rotation frequency by about 75% was observed. The presence of a relatively strong third harmonic mode was considered to be the most important clot indicator.

In the following, the flow in an ECMO circuit using a centrifugal pump will be considered. A laboratory setup has been used to further improve our understanding of the fluid mechanical aspects of the flow, along with its relation to the generated sound that may be registered near the pump. The results will not be presented in detail herein, but the knowledge gained will be used to explain our findings. An acoustic approach to detect thrombi has been developed and applied to data gathered at the ECMO center at Karolinska University Hospital, Solna, Sweden. It will be shown that the presence of blood clot can be identified by considering the low-end of the frequency spectrum, within the range of infrasound (i.e., below 20 Hz). For the seven patients that were observed, this approach detected all cases with blood clots and no false positive cases were found. Naturally, due to the low number of cases studied, more cases should be considered to further support the approach. Moreover, with the theoretical background based in fluid mechanics, we provide outlining rationale for the feasibility of the proposed method.

MATERIALS AND METHODS

Clot detection has been studied experimentally, both at "bedside" (at the ECMO Center at Karolinska University Hospital, Solna) and in a laboratory environment (Department of Mechanics, KTH). The laboratory experiments have been used to improve our understanding of the flow in a centrifugal ECMO blood pump (CentriMag, Thoratec, Pleasanton, CA, USA) and the tubing leading to and from it. The experimental setup is a replica of the clinically used system. However, the fluid used in the experimental system consists of standard 0.9% NaCl water solution instead of blood.

The bedside measurements were performed on seven patients hospitalized at the ECMO unit during a 5-week period. The patients had different age, diagnosis, and sex. No distinction was made between the veno-arterial and the veno-venous ECMO configuration. Clinical differences between the patients were not considered. The study focused solely on the registered acoustic signals. The spectra of the acoustic signals were compared to the clinical observations made by the treating team and the pumps were examined to elucidate the presence of thrombus when possible, that is, after removal from the ECMO circuit. Observations of the blood carrying tubes leading to and from the pump were also carried out using a flashlight to illuminate and detect possible clots.

The bedside sound acquisition was made passively, near the tubing leading to and from the pump. No interference with the treatment or handling of the patients took place. The ECMO system was configured according to the needs of the patients, independent of the measurements. For some of the patients, the pump was replaced. For example, one patient had three pump replacements during treatment. Three different types of recording devices were used, including a pair of microphones, a hydrophone, and an electronic stethoscope. The recorded acoustic data were digitized by each system respectively, as described below. The microphones (model 4189-A-021, Bruel & Kjaer, Nærum, Denmark) have a frequency range between 20 and 20 000 Hz, and were found to be less appropriate to be used with the method proposed herein. Equally, the 3M Littmann 3200 electronic stethoscope (3M, St. Paul, MN, USA) was also used to gather sound data, but it is not appropriate for recording the low-frequency data below 20 Hz. Hence, all results herein are based on data gathered by a hydrophone (model 8103, Bruel & Kjaer). It has a frequency range of 0.1 Hz to 180 kHz, and a sensitivity of 1 V/μPa. The hydrophone was connected through an amplifier (model 2635s, Bruel & Kjaer) to a computer storing the digitized signal in *wav* format (sampling rate of 44.1 kHz and digitized with 16 bits). The setup allowed for simultaneous recording from the different devices that in turn enabled differences in frequency response to be identified.

The acoustic signals were acquired over periods of 10, 30, and 60 s. The longest sampling time was used to assess the variability within the measured signal. This variability was found to be low. Therefore, only 10 and 30 s sampling time was used for the data analysis. Theoretically, the 10 s sample period should be adequate for capturing modes with 1 Hz and above. However, in a noisy environment (such as at a hospital), the longer sampling time yields clearer spectra.

The spectral content of the gathered data was analyzed with Matlab routine *pwelch* (MathWorks, Natick, MA, USA). The circuit was determined to contain a clot if a significant low-frequency component (i.e., above a threshold level) was present in the spectrum. This threshold was set to about two orders of magnitude larger than the corresponding low-frequency component in a clean laboratory ECMO circuit (Fig. 1, left frames). This threshold requires an amplification of the low-frequency

TABLE 1. *A summary of the different cases, follow-up duration, typical pump rotation rate, and typical flow rate. The two right columns indicate if clot was observed clinically and/or by the acoustic signal, respectively*

Patient	# Days	RPM	LPM	Clinically observed clot	Clot detected from acoustic signal
1	1	3200	2.5	No	No
2	3	2400	2	No	No
3	6	2200	0.35	Suspected	Yes
4	18	3800	4	Yes	Yes
5	6	2100	0.25	Yes	Yes
6	5	2000	0.25	Yes	Yes
7	1	4300	4.85	Suspected	Yes

signal due to a clot, large enough to be in the order of or greater than the dominating frequency without a clot, most often related to the pump rotation frequency. Moreover, in the no clot situation, the amplitude of the low-frequency signal is several orders of magnitudes smaller than all other dominant frequencies appearing in the spectrum.

RESULTS

A summary of the bedside results, including the different findings, is presented in Table 1. The operating conditions for the pump and the duration of the treatment are indicated as well as if a clot was detected by the medical team and/or by the acoustic measurements. Moreover, the two cases where the presence of a clot could not be confirmed but was suspected by the ECMO team are also indicated in Table 1. For Patients 1 and 2, both the proposed sound-acoustical approach and the clinical observations gave negative results (i.e., no clot). For Patients 3 and 7, the occurrence of a blood clot was suspected clinically whereas the acoustic method gave a positive result. For Patients 4–6, both the clinical and the acoustical results showed presence of a clot, verified through the presence of blood clot in the pump after removal (Patient 5).

In the following, two selected cases are described in some more detail. Only the results from the hydrophone are presented as the microphones and the stethoscope used in this study are unable to capture the low-end of the spectrum. First, we compared the signal from a laboratory setup running at 3300 rpm and an adult patient with the pump running at 3200 rpm and a flow rate around 2.5 liters per minute (LPM). Figure 1 depicts the spectrum at the inlet and outlet of the pump. The spectrum is normalized by the amplitude of the peak frequency associated with the pump rotation rate. In the laboratory (clean) setup, within the depicted range of frequencies, the pump rotation frequency and its first harmonic can be noted. Similarly, the two strongest signals in the bedside case are related to the corresponding frequencies. Additionally, four low amplitude peaks at low frequencies between 1 and 5 Hz can be observed. These peaks are more than two orders of magnitude smaller than the amplitude of the pump rotation frequency. These low-frequency modes that are weaker in the clean system, are amplified by the presence of red blood cells. This amplification is even stronger in the presence of clots (i.e., larger particles) as has been observed for Patients 3–7 in Table 1. The threshold set by us in this work requires that the amplitude of the low-frequency signal is large enough to be in the order of or greater than the dominating frequency without a clot, most often related to the pump rotation frequency. Low-frequency signals above the threshold are being considered as indicative for a clot. For Patients 1 and 2, we observed low-frequency signals that are one order of magnitude smaller than the threshold, and therefore, these cases are considered as clot-free. Moreover, no clinical indications for blood clot were observed for these cases. Patient 3 is an infant with the pump operating at a rotation rate of approximately 2200 rpm (36.67 Hz) and a flow rate of around 0.33 LPM. However, it needs to be noted that the pump rotation rate and the flow rate were varied during the treatment of the patient. The spectra of two recordings are displayed in Fig. 2. The first recording was made during the early stages of the treatment and the second recording 5 days later, just before the patient was removed from the ECMO circuit. Already in the early recording, a clear peak is evident at approximately 2 Hz. This peak is larger by more than an order of magnitude as compared to the peak due to the pump rotation rate at both inlet and outlet tubes. A clear harmonic of this signal (around 4 Hz) is also observed. These low-frequency peaks are also present in the recording 5 days later. The results indicate that a blood clot was present already during the early recording. No clinical notes have been made with respect to the presence of clots.

This case (Patient 3) also shows a relatively strong artifact in the form of a peak at 50 Hz. The frequency of this peak is related to that of the power installation

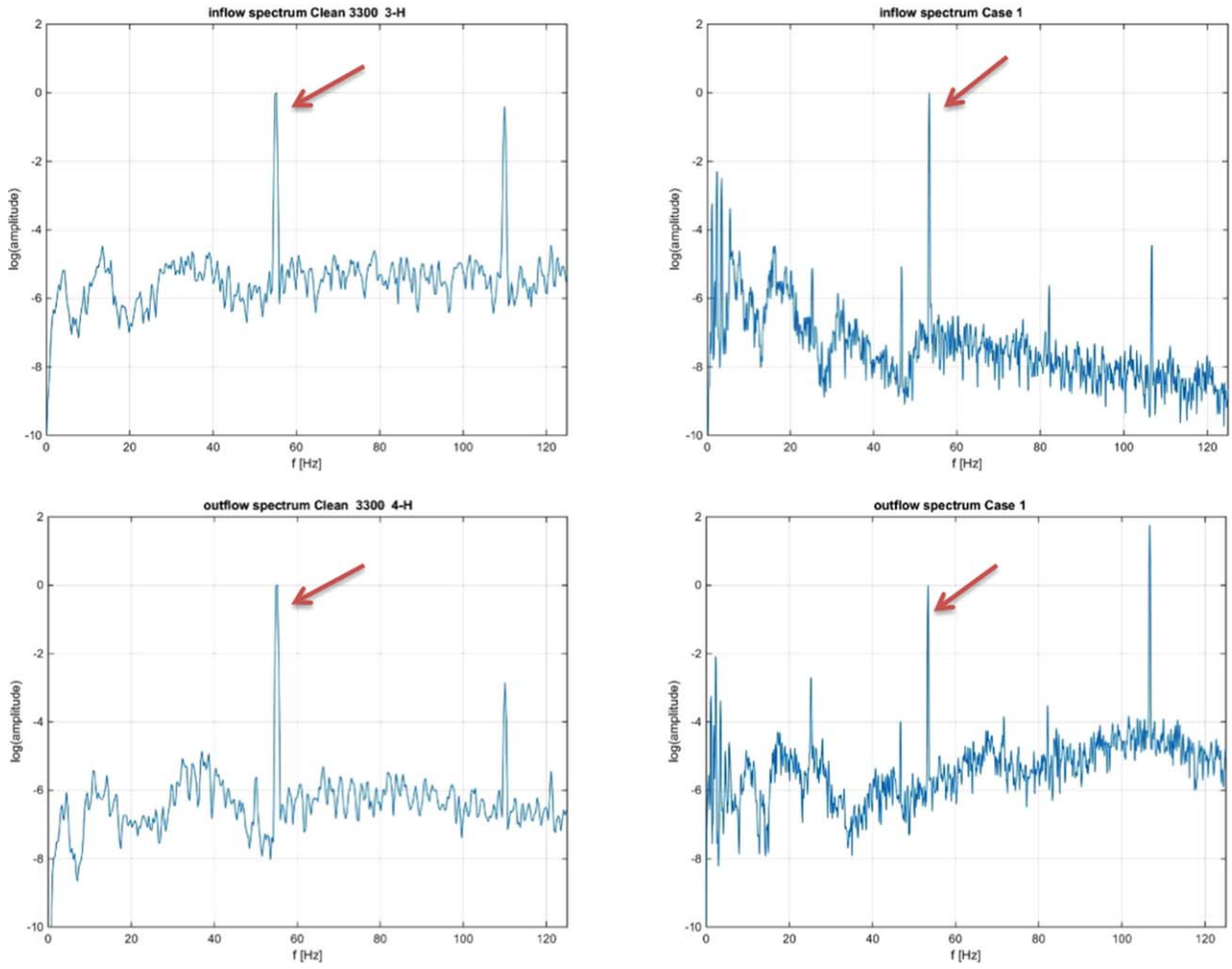

FIG. 1. The inflow (upper-left) and outflow (lower-left) spectrum of a clean pump in a laboratory ECMO circuit. The corresponding spectra for bedside Patient 1 are depicted in the upper-right and lower-right frames. The spectrum is normalized by the amplitude at the rotation rate frequency (3200 rpm = 53.3 Hz, for the right frames). The pump rotation frequency is marked by an arrow and is used for normalizing the spectrum. The semi-log of the spectrum is used to show the large variation in the strength of spectral components. The low-frequency signal at a few Hz is smaller by more than four and two orders of magnitude as compared to the signal due to the pump rotation rate for the clean and the bedside ECMO circuits, respectively. As the low-frequency component is smaller by more than one order of magnitude than the normalizing signal, we define this Patient 1 to be *clinically* clot-free. [Color figure can be viewed at wileyonlinelibrary.com]

and the amplitude depends on the environment and possible suppression by the sensor device. For example, the microphones used in this study do suppress this artifact. The presence of this frequency may be problematic if the pump rotation rate is a multiple of 25 Hz (1500 rpm) or 50 Hz (3000 rpm) and if the clot detection is based on harmonics of the pump rotation frequency. The spectrum for the other cases has been very similar also for pumps running at higher rotation rates. For example in Patient 7, the pump was running at 4300 rpm (71.67 Hz) and 4.85 LPM. The spectrum revealed that the strong modes at the inlet are found at very low frequencies: around 1.25 Hz accompanied by two harmonics.

The results given above do not give any indication to location, size, or the mechanism generating a clot. Therefore, a more detailed study has been initiated to understand the flow itself and the hypothesized interaction between the clot fragments (particles) and the flow. Flow visualizations in the lab setup revealed the presence of unsteady swirling flow both at the inlet and the outlet of the pump. In fact, in the presence of particles (such as clot fragments) depending on the particle size and density, resonance may occur leading to the amplification of the acoustic signal within the infrasound range.

DISCUSSION AND CONCLUSIONS

A method for detecting blood clots in the ECMO circuit has been proposed. The main idea is to study the relative strength of the low-frequency modes in

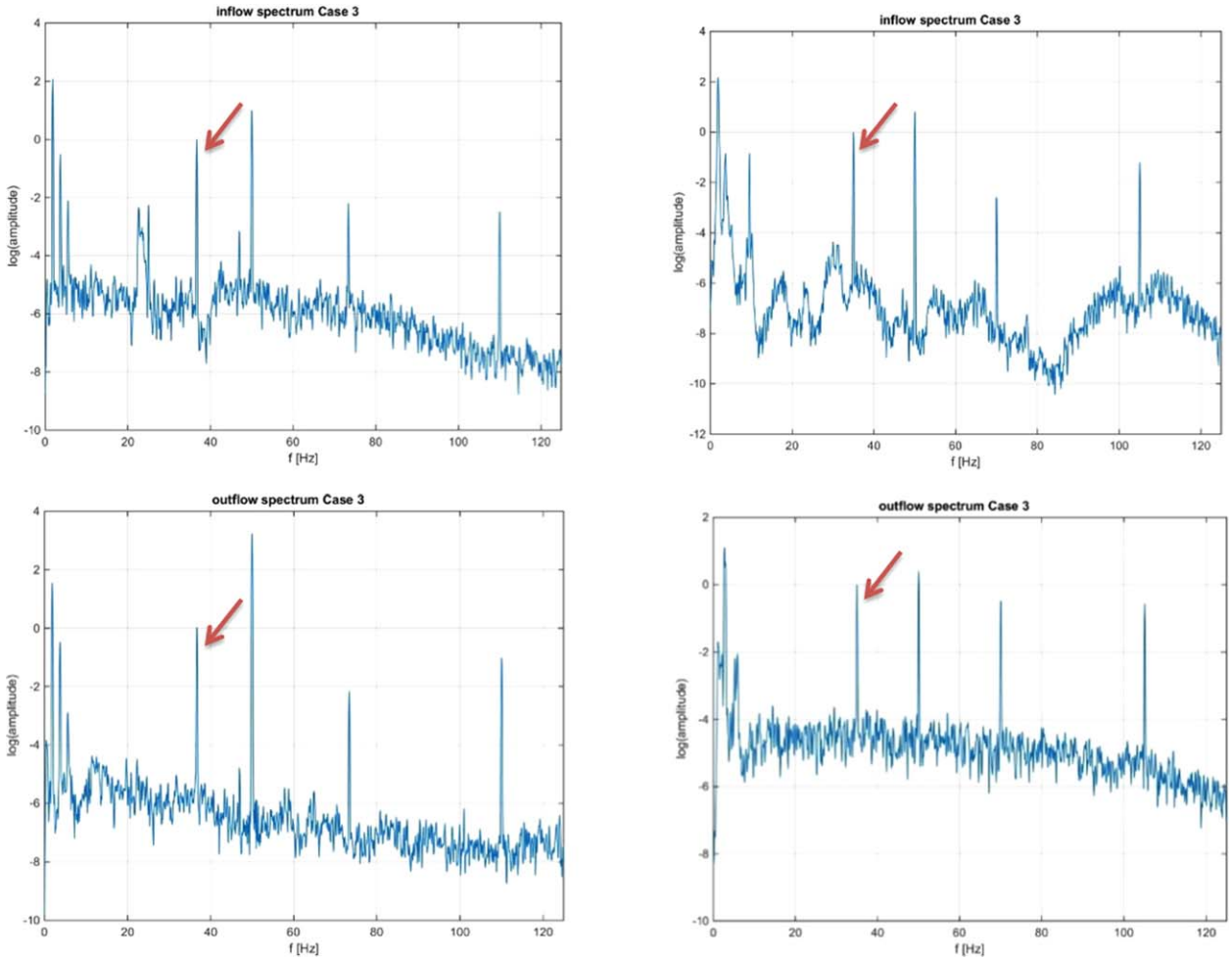

FIG. 2. Infant patient in the ECMO circuit (Patient 3). The upper and the lower frames are related to the inflow and outflow signals, respectively. The left and right frames are related to the same patient but the right frames were registered 5 days after the left one. The pump was running at 2200 rpm (36.67 Hz) and 0.33 LPM at the first registration and at 2100 (35 Hz) and 0.2 LPM in the later registration. The arrows mark the pump rotation rate used for normalization of the spectrum. Note the strong signal at about 2 Hz, which as in the earlier registration is two orders of magnitude stronger than the corresponding pump rotation frequency. In all frames, the clot threshold criterion is met with a wide margin and hence these registrations indicate a clear presence of a clot. Note also the strong signal at 50 Hz (power line frequency). [Color figure can be viewed at wileyonlinelibrary.com]

the acoustic signal at the inlet and outlet sections of the centrifugal pump. It has been shown that the clean circuit generates low-frequency (order of a few Hz) signals with an amplitude that is smaller by several orders of magnitudes as compared to the strongest signals. In circuits with clot, the low-frequency signal is of the same order or larger (by as much as two orders of magnitudes) compared to the normalizing signal. This wide separation range enables to introduce a quite safe threshold for eliminating false positive clot indication. Thus, the approach is different from previous studies performed on LVADs (cf. 9,10) as the analysis does not focus on the audible frequency range. The approach itself is non-invasive and does not require any modifications of the ECMO system. The only requirement is that the recording device used is able to handle low-frequency signals of the order of 1 Hz.

Our bedside study was limited to seven patients (some with several pump replacements) followed over a 5-week period. The study clearly shows that blood clots can be observed using the spectrum of the hydrophone signal. No clear false positives or negatives were found. Thus, it needs to be stressed that this work is a successful *proof of concept* and that further data must be acquired to determine the level of sensitivity and specificity of the diagnostic approach of using sound registrations.

The sound generated in the ECMO system can arise from mechanical and/or fluid mechanical sources. One possible source is flow field gradients (gradients of the so-called Lighthill tensor). Turbulence

generates sound through this mechanism, where the generated sound is of broadband character (covering a range in the spectrum). In contrast, the sound generated from a shear-layer or the wake behind an object may, under certain conditions, be modal (i.e., having a single frequency). Moreover, sound can be generated when the flow interacts with a moving object. All of the above mechanisms have been described by Ffowcs Williams and Hawkings (11). A review of the different mechanisms for sound generation may be found in Farassat (12).

In the ECMO system, one may identify several possible sources of sound. These include wakes behind objects (e.g., clot fragments), shear-layers, swirling flow regions, standing acoustic waves in the system, and sound generated by cavitation. Under fixed flow conditions, wakes and shear-layers generate pressure fluctuations which, when normalized by appropriate parameters, lead to constant or weakly flow dependent values. The frequency is commonly normalized by a characteristic velocity, U, and a length scale, D, yielding the so-called Strouhal number defined as $St = f \times U/D$, where f is the frequency of the fluctuations. In the case of a wake, the Strouhal number, St, has a value close to 0.2 for a wide range of flow speeds (Reynolds numbers). A similar analysis may be done for shear-layers and cavitation bubbles. Knowing the Strouhal number, it is possible to find the (modal) frequency associated with the flow. For cavitation bubbles, the analysis yields frequencies that are approximately four to five orders of magnitude greater than the frequencies found in this study. On the other hand, both wakes and shear-layers may lead to frequencies from one to a few 100 Hz. Thus, these two possible mechanisms of sound generation cannot be excluded.

As stated in the introduction, several research groups have previously proposed the use of sound and vibration generated by blood pumps to identify the presence of blood clots. None of these papers discussed the mechanism for the generation of the vibrations/sound. Without a plausible explanation of the underlying physical mechanism, it is unclear whether the observed changes in the acoustic signal are solely due to the presence of a blood clot or not. The setup and the results of Hubbert et al. (10) and Kawahito (13) indicate that the generated frequencies are of wake character. However, as the low-end resolution of the spectrum is limited, it is not possible to draw any further conclusions. In the work of Kaufmann et al. (9), the sound signature of HeartWare HVAD LVAD System is studied in vivo. The frequency response of the registering system was not stated. However, the spectra presented in the paper included low-frequency information. In figures 1 and 3 in the paper of Kaufmann et al. (9), it is observed that the lowest frequency modes are smaller than (about 7 Hz in their figure 1) or equal to (about 10 Hz in their figure 3) the pump rotation frequency. For the pump considered, the authors show that the most indicative condition for pump thrombosis is the existence of a third harmonic of the pump rotation rate, not present in the control group ($P < 0.0001$). It would be interesting to compare and assess this criterion to the criterion proposed here, namely the modes within the infrasound range.

The use of the low-frequency signal instead of a harmonic of the pump rotational rate is believed to be more appropriate for detecting blood clots. This belief is based on the theoretical argument that the clot interacts with the vortex core at the inlet/outlet of the pump and thereby amplifies the basic vortex signal. In our laboratory setup, a strong swirling flow at the inlet to the centrifugal pump was observed. The inlet swirl was characterized by an unsteady vortex core oscillating at a rate corresponding to a factor 3 to 5 times smaller than the pump rotation frequency. High-speed flow visualization showed that particles heavier than water may be captured in the central meandering vortex core and yield a strong signal in the spectrum for some pump rotation rates. A theoretical explanation of this phenomenon may be deduced from the numerical simulations of IJzermans et al. (14). In spite of the expected increase in the centrifugal force acting on the particles, the particles may be captured in a vortex core under certain conditions. It is believed that the observed strong low-frequency peaks are due to this effect. Thus, our future work will explore these hypotheses in more detail, both experimentally and theoretically.

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Discarded Livers Find a New Life: Engineered Liver Grafts Using Hepatocytes Recovered From Marginal Livers

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Abstract: Treatment for end-stage liver failure is restricted by the critical shortage of donor organs; about 4000 people die in the USA while waiting for a transplantable organ. This situation has been a major driving force behind the rise of tissue engineering to build artificial tissues/organs. Recent advancements in creating transplantable liver grafts using decellularized liver scaffolds bring the field closer to clinical translation. However, a source of readily available and highly functional adult hepatocytes in adequate numbers for regenerative liver therapies still remains unclear. Here, we describe a new method to utilize discarded livers to make transplantable new liver grafts. We show that marginal donor livers damaged due to warm ischemia could be treated with machine perfusion to yield 39 million viable hepatocytes per gram of liver, similar to fresh livers, and these cells could be used to repopulate decellularized liver matrix (DLM) scaffolds to make transplantable liver grafts. The hepatocytes from recovered livers sustained their characteristic epithelial morphology while they exhibited slightly lower protein synthesis functions both in plate cultures and in recellularized liver grafts. The dampened protein synthesis was attributed to residual endoplasmic reticulum stress found in recovered cells. The results here represent a unique approach to reengineer transplantable liver grafts solely from discarded organs. **Key Words:** Machine perfusion—Hepatocytes—Liver recellularization—Endoplasmic reticulum stress.

Treatment for end-stage organ failure is restricted by the critical shortage of donor organs with the organ waiting list currently at 123 000 requests, a number that far exceeds the supply of available organs and that continues to grow by 5% each year. This situation has been the major driving force behind the rise of whole-organ engineering that aims to build transplantable organ substitutes to address the void in organ replacement therapies (1). One limiting aspect in whole-liver engineering is the lack of a reliable cell source for primary adult hepatocytes. Pluripotent stem cells have the potential to generate an abundant supply of functional hepatocytes for use in cell-based therapies (2). However, while a number of protocols have derived "hepatocyte-like" cells from a pluripotent state, prohibitive limitations such as low yield and incomplete maturation still persist (3). Moreover, the protocols that describe the generation of the differentiated cells take 2 to 4 weeks, substantially increasing the time, cost, and

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