International Journal of Chemical and Physical Sciences, ISSN:2319-6602
IJCPS Vol. 4 Special Issue ETP – 2015
www.ijcps.org

Effect of Low Frequency Mechanical Vibrations on Human Blood (in vitro)

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Abstract

The activities like excessive power house machine vibrations, uncontrolled blasting, marathons, karate, operating a dumper truck, hand transmitted vibration from powered tools like drilling machines, riding in a bus or driving a truck etc. cause excessive mechanical stress in circulation of human body. Due to excessive mechanical stress on human body, red blood cells are at risk of developing disorders. When a human body or a part of the body is exposed to excessive mechanical vibrations, fragmentation of red blood cells (RBC) or hemolysis may occurs. Damaged RBC causes change in blood viscosity / surface tension and thus blood circulation. In the present study, the effect of mechanical vibrations on human whole blood (in vitro) were studied on the basis of biochemical study, hemolysis using single wavelength of 540 nm absorption, chemical bond studies by using Fourier Transform Infra Red (FTIR) spectroscopy with spectral range 400 - 4000 cm^{-1}, and morphological study by using Scanning Electron Microscopy (SEM). Mechanically vibrated human whole blood samples were compared to non vibrated whole blood sample. Electrodynamic shaker from M/s. Spectral Dynamics, USA, was used for generation of sinusoidal mechanical vibration in the frequency range of 10 -50 Hz, and displacement of 10 mm. Vibration test system consists of shaker, power amplifier, control system including data analysis software, accelerometer, charge amplifier and cooling blower. Human whole blood was mechanically vibrated at different frequencies ranging from 10 Hz to 50 Hz with amplitude of 10 mm for exposure 10 minute time. Vibrated and non vibrated whole blood were compared on the basis of biochemical study, hemolysis, Fourier Transform Infra Red (FTIR) spectroscopy with spectral range 400 - 4000 cm^{-1} and morphological study using SEM.

Keywords: Vibration; Frequency; Whole blood; Hemolysis; Morphology; FTIR.

Introduction

When two different fields namely vibration engineering and biology interact, extremely valuable and exciting new directions in research are created. Vibration is a mechanical phenomenon whereby oscillations occur about an equilibrium point. The oscillations may be periodic such as the motion of a pendulum or random such as the movement of a tire on a gravel road. Blood is a fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. The effects of mechanical vibrations on human blood were already reported. The increase of frequency from 10 to 20 Hz seems to induce damage on the RBC (Red Blood Cell) membrane and this effect was dependent on frequency and suggested precaution with the use of the vibration generated in oscillating platforms (OP) [1]. The effect of vibration on skin microcirculation was

studied to investigate the possibility of clinical use of vibration to prevent and treat pressure ulcers [2]. Of the many physical and biochemical changes, which occur during muscular activities, the study was carried out to evaluate the damage induced in red blood cells by exposure to impulse vibration [3, 4]. This study investigated the hemorheological characteristics of blood flow with applying vibration to a non-aggregating red blood cell suspension. To obtain the non-aggregation RBC suspension, blood samples were subjected to vibration at a specific condition, in which viscosities were measured before and after the treatment respectively [5]. Vibration under shear flow causes the reduction of flow resistance for shear-thinning fluids [6]. The study was carried to show how blood cells interact with each other and with the blood vessel wall [7]. A group of workers showing early changes in RBC metabolism were reported [8]. Effects of vibrations on human body by exercises, congadrum, and sports have been already reported [9-11]. Vibrated and untreated whole blood were compared on the basis of biochemical study, hemolysis, Fourier Transform Infrared (FTIR) spectroscopy with spectral range 400 - 4000 cm^{-1} and morphological study. In the present study, the effect of mechanical vibrations on human whole blood was studied on the basis of biochemical parameters, hemolysis using single wavelength of 540 nm absorption, chemical bond studies by using Fourier Transform Infrared (FTIR) spectroscopy with spectral range 400 - 4000 cm^{-1}, morphological study by using SEM.

Experimental Work

Blood samples

Blood was withdrawn from one 38 years old male at standard laboratory conditions. Blood samples of 9 ml each time were withdrawn in syringe and anticoagulant (2 ml of EDTA) was added. This blood sample was used in each experiment and more than three experiments were performed. 1.5 ml of blood was taken in a plastic viol tube and six samples for each experiment were prepared.

Electrodynamic Shaker

Blood samples were placed in plastic viol and fixed on the platform of Electrodynamic shaker, which is shown in figure 1. Electrodynamic shaker from M/s. Spectral Dynamics, USA, was used for generation of sinusoidal mechanical vibration in the frequency range of 10 - 50 Hz, and displacement of 10 mm. Vibration test system consists of shaker, power amplifier, control system including data analysis software, accelerometer, charge amplifier and cooling blower. The drive signal from the digital signal processing controller is input to the amplifier. The amplifier takes the low voltage (0 - 5 volts) and milliamps current from the controller and converts it to higher voltages and currents (0 -100 volts and hundreds or thousands of amps) for the shaker. The moving element (armature) of the shaker is suspended in a strong magnetic field. The current from the amplifier flows through the wire in a magnetic field. The

suspended moving element is restrained and allowed to move only up and down. The movement of the platform is sensed by the piezoelectric sensor. The conditioned signal is fed into the closed control loop from the charge amplifier to a pre-defined reference level. The control loop adjusts the drive signal to the amplifier to maintain the reference level. Sinusoidal current produces a sinusoidal motion, random currents produce random motion. Human whole blood was exposed to sinusoidal vibrations of low frequency i.e. 10 - 50 Hz, amplitude of 10 mm for 10 minute duration using Electrodynamic shaker system. Human whole blood without vibration treatment was considered as a control. Six numbers of blood samples of 1.5 ml each were taken for a single experiment in a set. Each set is exposed to mechanical vibrations of frequencies 10 Hz, 20 Hz, 30 Hz, 40 Hz and 50 Hz and one set without mechanical vibrations i.e. control. Each set of whole blood sample was placed on the platform of electrodynamic shaker as shown in figure 1. Electrodynamic shaker is generating controlled mechanical vibrations. Experiment was done for fixed amplitude of 10 mm with 10 minute duration. Blood samples with and without mechanical vibrations for all experiments were taken from same person and were kept in same environmental conditions. In the present study, the effect of mechanical vibrations on human whole blood was studied on the basis of biochemical parameters, hemolysis using single wavelength of 540 nm absorption, chemical bond studies by using Fourier Transform Infrared (FTIR) spectroscopy with spectral range 400 - 4000 cm^{-1} and morphological study by using scanning electron microscopy (SEM).

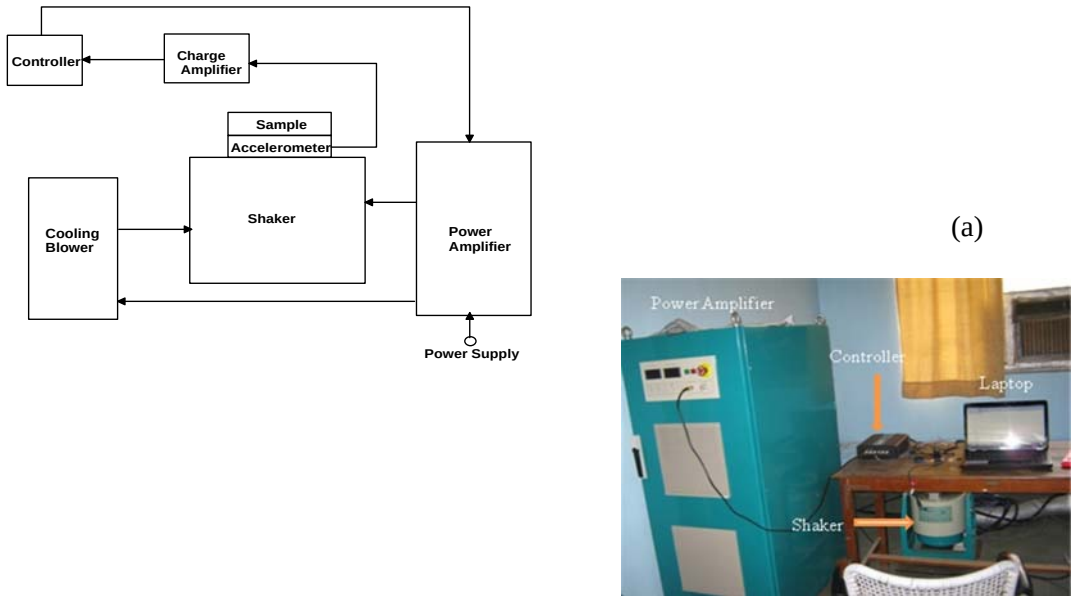

Figure. 1. (a) block diagram of electrodynamic shaker and (b) experimental setup for generation of controlled vibration.

Procedure

Three sets of tubes were used in each experimental study. First set of three tubes were subjected to 0 Hz i.e. non vibrated (control), the second set of three tubes were subjected to 10 Hz, the third set of three tubes to 20 Hz, the fourth set of three tubes were subjected to 30 Hz, fifth set of three tubes were subjected to 40 Hz and sixth set of three tubes were subjected to 50 Hz in an platform of electrodynamic shaker. EDTA mixed blood samples (1.5 ml) were distributed in plastic tubes. The tubes were under the platform of the electrodynamic shaker and fixed with adhesive tape. The amplitude of the vibration was kept fixed at 10 mm. After the fixation of the tubes, the one of the frequency was selected (10 to 50 Hz) and the time of exposure was kept constant at 10 min. This procedure was repeated for each frequency and each experimental set. In the control set, the tubes with blood were not subjected to vibrations on the platform.

Biochemical Studies

After the mechanical vibrations from 0 Hz to 50 Hz, the blood samples were given for lab test of hemogram to a standard biochemical laboratory.

Hemolysis

After the mechanical vibrations from 0 Hz to 50 Hz, the blood samples were centrifuged at 3000 rpm for 5 min. Supernatant of 1 ml volume was separated and the absorbance at 540 nm in a spectrophotometer (Perkin Elmer lambda - 950, Singapore) using 0.9 % NaCl as a blank was taken. The hemolysis was evaluated by dividing the absorbance of the supernatant of vibrated sample of blood (10 to 50 Hz) by the supernatant of the control sample (0 Hz).

Fourier Transform Infra Red (FTIR) spectroscopy

FTIR measurements were conducted in transmission mode and measured spectral range was 400 - 4000 cm^{-1}. It is important to study the blood components in terms of bio-molecular changes using FTIR spectroscopy. FTIR spectra of whole blood were studied for mechanical vibrations at different frequencies ranging from 10 Hz to 50 Hz with amplitude of 10 mm for exposure of 10 minute time and non vibrated (Control) blood. FTIR spectra of vibrated and non vibrated blood were compared.

Morphological study

Scanning electron microscope (JEOI JSM-6360A) was used to obtain RBC images before and after vibration. RBC samples for scanning electron microscope (SEM) were prepared by using the standard procedure. The SEM images of RBC were taken for different magnifications for vibrated samples (frequency 10 to 50 Hz with 10 mm amplitude and 10 minute exposure of duration)

Results and Discussions

Biochemical studies

Comparison of haemograms of vibrated blood samples with control is shown in Table No. 1. It

shows minor changes in hemoglobin, total WBC count, haemotocrit (HCT) etc.

Hemolysis

Comparison of Hemolysis of vibrated blood samples with control is shown in Table 2. It shows that changes in hemolysis is significant.

Fourier Transform Infrared (FTIR) spectroscopy

Comparison of Fourier Transform Infrared (FTIR) spectra of vibrated blood samples with control is shown in Table No. 3.

From Table 3, the prominent absorption peak 3352 cm^{-1} is due to the N-H stretching mode (amide A), for mechanically vibrated samples, the peaks shifted to 3295 cm^{-1}, 3333 cm^{-1}, 3298 cm^{-1}, 3360 cm^{-1} for 10 Hz, 20 Hz, 30 Hz, 40 Hz, 50 Hz frequencies respectively.

Table 1. Comparison of hemograms of vibrated blood samples with control

Test No.	Ref. Range, Unit	Frequency, Hz					
		0	**10**	**20**	**30**	**40**	**50**
1	14-18 gm %	14	14.3	13.9	14.8	13.9	13.9
WBC Count							
2	(4000 - 11000) /cmm	9500	10100	9600	9600	10100	9800
3	40 - 70, %	66	63	63	67	65	67
4	20 - 45, %	23	25	26	24	26	24
5	1.0 - 6.0, %	9	10	9	8	8	8
6	0 - 8, %	2	2	2	1	1	1
7	0 -1, %	0	0	0	0	0	0
RBC Indices							
8	40 - 54, %	52.2	55	51.8	55.4	52.5	51.6
9	4.5 - 6.5 mil./cmm	6.06	6.34	6.02	6.27	5.96	5.84
10	76 - 96	86.2	86.8	86.2	88.5	88.1	88.4
11	27 - 32	23.1	22.5	23	23.6	23.3	23.8
12	32 - 36	26.8	26	26.8	26.7	26.4	26.9
Platelets indices							
13	1.5 - 4.5	2.56	2.42	2.25	2.35	2.68	2.35

[In Table no. 1, Test no. 1: Haemoglobin, 2: Total WBC count, 3: Neutrophil, 4: Lymphocytes, 5: Eosinophil, 6: Monocytes, 7: Basophil, 8: Haemotocrit (HCT), 9: RBC, 10: MCV count, 11: MCH, 12: MCHC, and 13: Platelet count]

Table 2. Comparison of Hemolysis of vibrated blood samples with control

Frequency(Hz)	Abs. at 540 nm	Hemolysis
Control	0.2127	-
10	0.1672	0.79
20	0.6274	2.95
30	0.2405	1.13
40	0.1845	0.87
50	0.1527	0.72

Table 3. Comparison of FTIR spectra of vibrated blood samples with control

Control		10 Hz		20 Hz		30 Hz		40 Hz		50 Hz		group
Band	% T	Band	% T	Band	% T	Band	% T	Band	% T	Band	% T	
3352	14.53	3295	17.11	3333	14.73	3298	17.97	3368	14.14	3360	13.82	1
-	-	2966	63.21	2967	66.49	2958	61.79	2970	64.99	2966	66.77	2
-	-	2879	75.41	2871	79.59	2871	75.83	-	-	-	-	3
1637	56.10	1649	37.33	1641	51.91	1649	40.14	1638	57.25	1649	56.65	
1538	75.85	1544	51.00	1546	71.23	1535	55.03	1540	80.16	1536	76.60	4
1329	86.66	1361	75.41	1370	80.16	1361	73.68	1365	73.32	1361	77.19	5
1245	88.02	1221	79.57	1223	83.44	1230	77.74	1202	76.29	1212	78.38	6
1106	89.39	1082	86.99	1052	89.99	1082	88.55	1049	92.36	1047	91.77	7

[Functional group: 1: Amide A: N-H stretching of secondary amide proteins, 2: CH_3 asymmetric stretch: mainly lipids, 3: CH_3 symmetric stretch: mainly proteins, 4: Amide I: mainly C=O stretching of proteins, 5: Amide II: N-H bending and C- N stretching of proteins, 6: CH_2 bending: mainly lipids, 7: C-O stretch]

Bands observed at 1538 cm^{-1} in control, whereas for mechanically vibrated samples at 1544 cm^{-1}, 1546 cm^{-1}, 1535 cm^{-1}, 1540 cm^{-1}, 1536 cm^{-1} for 10 Hz, 20 Hz, 30 Hz, 40 Hz, 50 Hz frequencies respectively are due to Amide I mainly C=O stretching of proteins. Bands observed at 1329 cm^{-1} in control, whereas for mechanically vibrated samples at 1361 cm^{-1}, 1370 cm^{-1}, 1361 cm^{-1}, 1365 cm^{-1}, 1361 cm^{-1} for 10 Hz, 20 Hz, 30 Hz, 40 Hz, 50 Hz frequencies respectively are due to Amide-II N-H bending & C-N stretching of proteins. Bands observed at 1245 cm^{-1} in control, whereas for mechanically vibrated samples at 1221cm^{-1}, 1223cm^{-1}, 1230 cm^{-1}, 1202 cm^{-1}, 1212 cm^{-1} for 10 Hz, 20 Hz, 30 Hz, 40 Hz, 50 Hz frequencies respectively are due to CH_2 bending mainly lipids. Bands observed at 1106 cm^{-1} in control, whereas for mechanically vibrated samples at 1082cm^{-1}, 1052 cm^{-1}, 1082cm^{-1}, 1049cm^{-1}, 1047cm^{-1} for 10Hz, 20Hz, 30Hz, 40Hz, 50Hz frequencies respectively are due to C-O stretch of glucose region.

Morphological study

Figure 2 shows comparison of SEM images of vibrated blood samples with control.

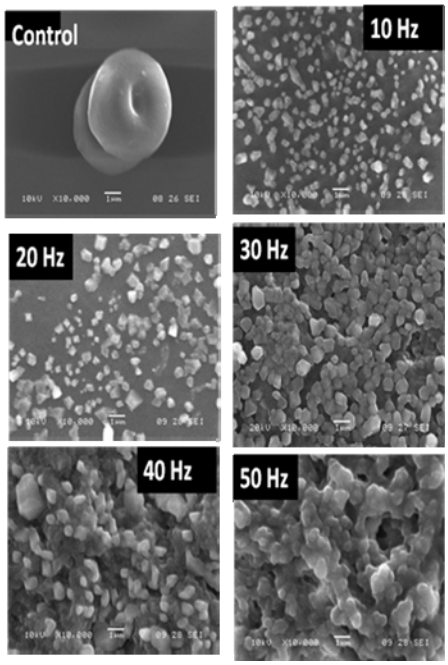

Figure 2. SEM images of vibrated RBC samples with control for morphological study

Figure 2 shows intact red blood cell was detected before exposure to mechanically vibrated samples i.e. control. Disordered states & shapes of red blood cells were seen after exposure to all of the mechanically vibrated samples i.e. 10, 20, 30, 40, & 50 Hz frequencies.

Conclusions

- Mechanical vibrations from 10 Hz to 50 Hz frequencies affect the whole blood samples and morphology of Red Blood Cell (RBC).
- Hemolysis of RBC is observed due to mechanical vibrations.
- Very minor changes are seen in haemograms of blood samples vibrated at 10 - 50 Hz frequencies.
- FTIR spectra of whole blood show that peaks associated with functional groups N-H stretching, C=O stretching, N-H bending and C-N stretching, CH_2 bending and C-O stretch are shifting due to mechanical vibrations. However, as the whole blood is composed of white, red cells and platelets, exact correlation of peaks is not clear.
- Vibrated RBC samples show disordered shapes and stacking due to mechanical low frequency vibrations.

Acknowledgement

Authors are grateful to Director, Central Water & Power Research Station, Pune, Director, School

of Basic Medical Sciences, Department of Physics, Savitribai Phule Pune University, Pune, and Head, Department of Physics, Savitribai Phule Pune University, Pune, for providing the research facilities to present work.

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