Research article

The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors and fibrin architecture of a Leukocyte- and Platelet-Rich Fibrin (L-PRF) clot and membrane. Part 1: evaluation of the vibration shocks of 4 models of table centrifuges for L-PRF

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Abstract

Background and Objectives. Platelet concentrates for surgical use (Platelet-Rich Plasma PRP or Platelet-rich fibrin PRF) are surgical adjuvants to improve healing and promote tissue regeneration. L-PRF (Leukocyte- and Platelet-Rich Fibrin) is one of the 4 families of platelet concentrates for surgical use and is widely used in oral and maxillofacial regenerative therapies. The objective of this first article was to evaluate the mechanical vibrations appearing during centrifugation in 4 models of commercially available table centrifuges frequently used to produce L-PRF.

Materials and Methods. The 4 different tested centrifuges were the original L-PRF centrifuge (Intra-Spin, Intra-Lock, the only CE and FDA cleared system for the preparation of L-PRF) and 3 other laboratory centrifuges (not CE nor FDA cleared for L-PRF): A-PRF 12 (Advanced PRF, Process), LW - UPD8 (LW Scientific) and Salvin 1310 (Salvin Dental). Each centrifuge was opened for inspection, two accelerometers were installed (one radial, one vertical), and data were collected with a spectrum analyzer. Each centrifuge was tested in 2 configurations (full-load or half load with 9ml blood collection tubes filled with water) and at the following rotational speeds: 1500, 1800, 2100, 2400, 2700, 3000 and 3300 rpm. Extra rotational speeds were used on some centrifuges. One centrifuge (Salvin) had only one available rotational speed (3400 rpm). For each test, the software documented both radial and vertical vibration.

Results. Very significant differences in the level of vibrations at each rotational speed were observed between the 4 tested machines. The original L-PRF centrifuge (Intra-Spin) was by far the most stable machine in all configurations. At the classical speed of production of L-PRF, the level of undesirable vibration on this centrifuge is between 4.5 and 6 times lower

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than with other centrifuges. Moreover, Intra-Spin always remains under the threshold of resonance, unlike the 3 other tested machines.

Discussion and Conclusion. Each centrifuge has its clear own profile of vibrations depending on the rotational speed, and this may impact significantly the characteristics of the PRP or PRF produced with these devices. This result may reveal a considerable flaw in all the PRP/PRF literature, as this parameter was never considered. It is now necessary to evaluate the impact of the vibration parameter on the architecture and cell content of the L-PRF clots produced with these 4 different machines.

Keywords. Blood platelets, growth factors, leukocytes, platelet-rich plasma, regenerative medicine, wound healing.

1. Introduction

Platelet concentrates for surgical use (Platelet-Rich Plasma - PRP and Platelet-Rich Fibrin - PRF) are blood extracts frequently used nowadays in many medical fields **[1]**, particularly in oral and maxillofacial surgery **[2,3]**, plastic surgery **[4]** and sports medicine **[5,6]**. The objective of all these technologies is to extract (through centrifugation and various handling methods) from a blood sample all the elements that could be use to improve healing and promote tissue regeneration **[7]**, particularly: the platelets (rich in growth factors)**[8]**, the fibrin (serving as a supporting matrix)**[9]** and in some cases the cell content (mostly the various populations of leukocytes)**[9]**. The literature on these products is quite confusing and controversial due to the lack of proper characterization of these many different products **[10,11]**. Recently, they were classified in 4 main families, based on their fibrin and cell content **[11-13]**: the 2 types of Platelet-Rich Plasma (PRP) are platelet suspensions that can jellify into a light fibrin gel after activation (Pure Platelet-Rich Plasma P-PRP, or Leukocyte- and Platelet-Rich Plasma L-PRP, respectively without or with leukocytes). On the contrary, the 2 types of Platelet-Rich Fibrin (PRF) only exist in a strongly polymerized fibrin gel form (Pure Platelet-Rich Fibrin P-PRF, or Leukocyte- and Platelet-Rich Fibrin L-PRF, respectively without or with leukocytes).

In oral and maxillofacial surgery, the use of PRPs is nowadays very scarce, as these various technologies are quite complicated, heavy to use in daily practice, expensive and offering quite mixed clinical results **[2,3]**. On the contrary, the L-PRF family developed very quickly these last years, as this technique is very simple and useful in daily practice, user-friendly and inexpensive **[11]**. Interesting results in vitro **[14,15]** and in some clinical oral applications were already published **[16-20]**, particularly during sinus-lift implant surgery **[21-23]** and gingival healing **[24-26]**. In this open-access method, a 9mL blood sample is taken without anticoagulant and centrifuged immediately during 12 minutes. At the end of the process, a L-PRF clot can be collected in the middle of tube **[9]**. This clot gathers almost all the platelets and half of the leukocytes of the initial blood harvest (with a majority of lymphocytes)**[9]**, and is an active source of growth factors release **[8,27]**. This clot can then be used directly as filling material or mixed with bone grating material **[2]**, or it can compressed into a strong fibrin L-PRF membrane, using an adequate surgical box designed to prepare it without damage (nowadays marketed with CE/FDA clearance as Xpression kit, Intra-Lock, Boca-Raton, FL, USA)**[28]**.

Even if the method is open-access, the early developers carefully optimized this technique, in order to get the best possible and most reproducible clots, membranes and finally clinical results **[28]**. Based on a slow learning through the feedback of the field experience and research, the protocol was tailored by using a high-quality table centrifuge,

specific glass-coated plastic tubes and a specific protocol (12 minutes, 2700 RPM). The relevant literature on the L-PRF was produced using this adequate material since more than 10 years **[2]**. The original open-access method and associated devices used since the early phases of the development of this technique are nowadays marketed with CE/FDA clearance as the Intra-Spin L-PRF system and kit (Intra-Lock, Boca-Raton, FL, USA). This inexpensive system is actually the only L-PRF system available on the market with all certifications and using the original protocol and devices.

Unfortunately, other authors without this experience decided to extrapolate the original L-PRF protocol with cheaper low-quality devices or by modifying some parameters of the initial protocol to adapt to the various centrifuges they decided to use **[29-31]**. The L-PRF clots and membranes produced with these modified materials and methods appeared obviously different (weight and volume of the clots for example) from the original L-PRF method, but this simple observation was often neglected. This chaotic situation of random modification of the protocol was already widely observed in the fields of PRP **[12]**, and it was often advocated that the mixed and controversial results and the absence of consensus in the use of PRP (particularly in sports medicine) was the consequence of this absence of control, characterization and evaluation of the methods and types of PRP in the literature. Some debates appeared in the L-PRF literature **[29-31]**, in order to highlight this absolute need to respect the original protocol and material, or at least to define clearly any variations of the L-PRF protocol/material as a different protocol **[28]**. It was advocated that changes in materials and/or protocols may affect considerably the L-PRF clot content and architecture **[25]**, and must be therefore characterize separately as a specific PRF-like product **[28-31]** and not as the original L-PRF described in the literature. This debate is very important, in order to avoid to create confusing data in the literature that may affect the credibility of the L-PRF technique because of inaccurate articles produced with inadequate methods or materials.

The exact differences between the various materials and methods to produce L-PRF and the characteristics of the different L-PRF products were not clearly demonstrated and published scientifically up to now. The characteristics of the centrifuges are important parameters to start with. All platelet concentrates are produced through the use of a centrifuge. As mechanical instruments, all centrifuges have specific mechanical characteristics that differ significantly among the many possible available models. However, these different characteristics were never evaluated before in the production of PRP/PRF. In the case of small table centrifuges used for L-PRF production, the most relevant parameters to evaluate appeared logically to be the vibrations of the centrifuges during the centrifugation process, the vibration shocks during the acceleration phases and an eventual resonance of the vibrations. All these mechanical characteristics may interfere with the quality and biological signature of the final L-PRF product.

The objective of this series of 3 articles was to point out the impact of the centrifuge characteristics and centrifugation protocol on the cell, growth factors and fibrin architecture of a L-PRF clot and membrane. In this first article, the mechanical vibrations (both radial and vertical) appearing during centrifugation were evaluated in 4 models of commercially available table centrifuges frequently used to produce L-PRF.

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2. Materials and methods

2.1. Description of the tested centrifuges

In this study, 4 different centrifuges, found on the market and used to produce L-PRF, were tested **(Figure 1)**. The country of manufacture being used by some companies as a claim for quality, the country of manufacture of each centrifuge and its main components was checked. The 4 selected centrifuges were purchased from their manufacturers (or distributors).

The first centrifuge was the original centrifuge used during the early development of the L-PRF open-access method and is nowadays marketed under the name Intra-Spin L-PRF centrifuge (Intra-Lock International, Boca-Raton, FL, USA; Made in Germany). It is actually the only CE marked and FDA cleared system for the preparation of L-PRF clots.

The 3 other centrifuges are not CE/FDA cleared for L-PRF, but they can be found relatively frequently available on the market for this use (mostly because they are much cheaper): centrifuge A-PRF 12 (Advanced PRF, Process for PRF, Nice, France; Country of manufacture not indicated on the label, components inside show “Made in China”), centrifuge LW - UPD8 (LW Scientific, Lawrenceville, GA, USA; Components made in China, assembled in the USA) and centrifuge Salvin 1310 (Salvin Dental Specialties, Charlotte, NC, USA; Made in China).

Figure 1. The 4 centrifuges used to produce L-PRF clots and tested in this study. From left to right: original L-PRF centrifuge (Intra-Spin, Intra-Lock), A-PRF 12 (Advanced PRF, Process), Salvin 1310 (Salvin Dental) and LW - UPD8 (LW Scientific).

2.2. Protocol of analysis of the vibrations

Each centrifuge was loaded with 8 blood collection tubes (Serum Vacuette 9 ml, Greiner Bio-One GmbH, Kremsmünster, Austria) filled with water to the manufacturer’s recommended level (approximately 9ml). The tube weights were measured on a high precision balance device (Sartorius M-Prove high precision balance, Model AY123, Sartorius AG, Goettingen, Germany) to ensure that each tube had a substantially equivalent load of water (full tube weights were measured between 18.41 and 18.43 grams).

Each centrifuge was opened for inspection and the placement of two accelerometers (Wilcoxon: Model 780A-IS, 100mV/g, Meggitt, Germantown, MD, USA). One accelerometer was used to access radial vibration on the centrifuges when under load and under

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acceleration. This radial accelerometer was positioned directly on the motor frame of each centrifuge, as close as possible to the bottom of the rotating tube holder. The other accelerometer was used to determine vertical vibration when under load and under acceleration. This vertical accelerometer was positioned on the centrifuge base, as close as possible to the lower edge of the rotating tube holder. The data were collected with a spectrum analyzer-FFT (Fast Fourier Transform) capable and its data processing software (Commtest Model VB7 and software Ascent 2013 Level 2, R3, v13.5.5; Commtest, GE Energy, Christchurch, New Zealand).

Each centrifuge was tested with two configurations: half tube load (3 or 4 tubes depending on capacity) and full tube load (6 or 8 tubes depending on capacity). For each configuration (half tube load and full tube load), tests were run at the following rotational speeds: 1500, 1800, 2100, 2400, 2700, 3000 and 3300 rpm. Extra rotational speeds were used on some centrifuges. One centrifuge (Salvin 1310) had only one available rotational speed (3400 rpm). For each test, the software documented both radial and vertical vibration. Plotted curves showing vibration (m/s^2) vs. frequency (Hz) were obtained from this documentation and recorded.

3. Results

All plotted curves for the tested centrifuges demonstrated a high level of acceleration in a very narrow range of frequencies. These were centered on the excitation frequency (rotational speed). Almost no vibration at other frequencies was noted. Since the software has the capability of combining several curves on a single chart, we were able to obtain for each machine and for a given configuration (half-full or full) a set of curves from which we were able to derive an envelope curve showing the level of vibration vs. the rotational speed. Therefore, all envelope curves could be combined on a single and final chart: one chart for the half-full configuration and one chart for the full configuration. These 2 final charts allow us to compare easily all machines tested.

This experiment highlighted 2 clear results **(Figures 2 and 3)**. First, all centrifuges experienced an increase in the level of vibrations when the rotational speed was increasing. Second, very significant differences in the level of vibrations at each rotational speed were observed between the 4 tested machines. Each machine had its clear own profile of vibrations depending on the rotational speed. The test curves of the 4 machines never crossed. These results were observed for both experimental configurations (half or full tube load).

The original L-PRF machine (Intra-Spin) presented the lower level of vibrations at all speeds in both experimental configurations, and the increase of the vibrations remained very limited when the speed was increasing. This was clearly the most stable machine on this aspect. As this machine served for the development of the L-PRF protocol and significant literature, these values can serve a standard of comparison with other machines.

The LW centrifuge presented a very strong increase of vibrations when the rotational speed was increasing. The vibrations of this centrifuge are 4.5 times higher than the vibrations of the Intra-Spin centrifuge for the production of L-PRF (2700 rpm) in full load configuration, and the difference was even stronger in half load configuration (5.2 times higher).

The Salvin centrifuge offered only one speed of centrifugation (3400 rpm), what was therefore the speed used to produce L-PRF with it. The vibrations of this centrifuge were 6 times higher than the vibrations of the Intra-Spin centrifuge for the production of L-PRF in

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full load configuration, and the difference was a bit stronger in half load configuration (6.3 times higher).

The A-PRF centrifuge presented the strongest increase of vibrations when the rotational speed was increasing. The vibrations of this centrifuge were 6 times higher than the vibrations of the Intra-Spin centrifuge for the production of L-PRF (2700 rpm) in full load configuration, and the difference was even stronger in half load configuration (6.8 times higher).

The results of this study were very clear, and highlighted that each centrifuge had its own vibration profile, and that devices can have considerable differences in terms of intensity of the vibrations.

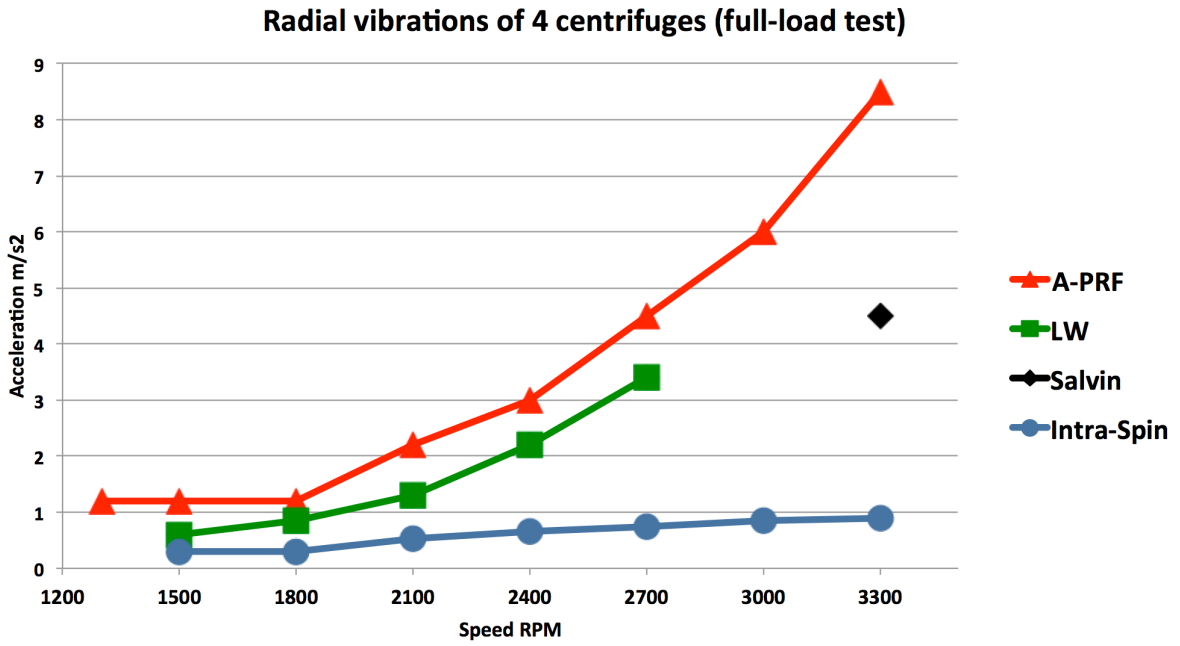

Figure 2. Radial vibrations of the 4 centrifuges during the full-load test. The curves showed the intensity of vibrations at each rotational speed (in RPM, Revolutions Per Minute). The lowest intensity of vibrations was observed always with the original L-PRF Intra-Spin centrifuge. At the classical speed of production of L-PRF, the level of undesirable vibration on the Intra-Spin centrifuge was between 4.5 and 6 times lower than with other centrifuges. Moreover, Intra-Spin always remained under the threshold of resonance, unlike the 3 other tested machines.

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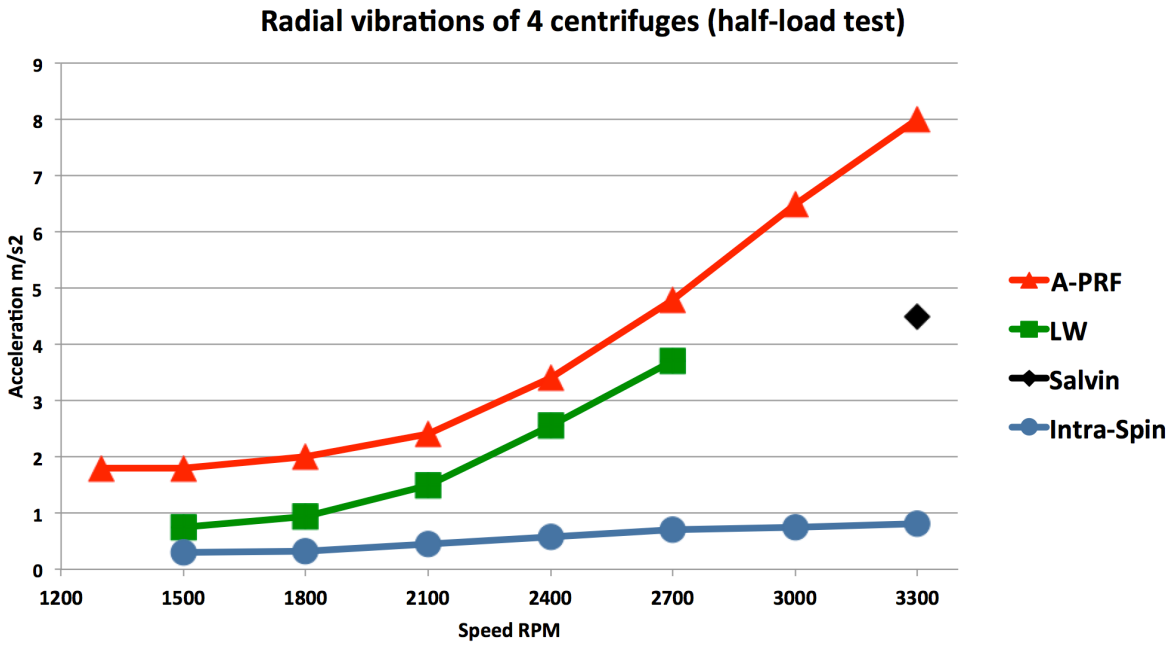

Figure 3. Radial vibrations of the 4 centrifuges during the half-load test. Half-load test was closer from the clinical reality, as clinicians often only use 3-4 tubes of L-PRF for a daily life small surgery. The curves showed the intensity of vibrations at each rotational speed (in RPM, Revolutions Per Minute). The lowest intensity of vibrations was observed always with the original L-PRF Intra-Spin centrifuge. At the classical speed of production of L-PRF, the level of undesirable vibration on the Intra-Spin centrifuge was between 5.2 and 6.8 times lower than with other centrifuges. Moreover, Intra-Spin always remained under the threshold of resonance, unlike the 3 other tested machines. The results were very similar to the full-load test, but the differences between machines were even more marked in this half-load configuration.

4. Discussion

Since the early phases of development of the L-PRF technology, scientists observed easily that the choice of the centrifuge and the protocol of centrifugation was affecting the final aspect (weight and size) of the L-PRF clot **[9,27]**. Good sense observations could reveal that the machines have different levels of vibrations, as it can be perceived easily by simply hearing them and placing a hand on the centrifuge during the centrifugation process. Despite this obvious scientific observation, no one investigated or even considered the quality of the centrifuge as an important parameter in the production of PRP and PRF. PRP are often produced with larger and heavier centrifuges than L-PRF **[27]**, and this may explain why this parameter was not investigated before. However, in the case of L-PRF, this parameter is very perceptible as the L-PRF technique was designed to be used easily in daily clinical practice and therefore with a small and light table centrifuge - therefore with a highest risk of vibrations and resonance during the centrifugation.

This study is the first research evaluating scientifically the intrinsic characteristics of the table centrifuges used to produce platelet concentrates for surgical use. It proves that the devices found on the market have very significant difference in terms of vibrations, and that all tested devices have much higher intensity of vibrations than the original L-PRF centrifuge (Intra-Spin). Moreover, when radial vibrations rise above 1, there is a serious risk that resonance occurs in the centrifuged tubes, what can provoke significant damage to the blood

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cell content of the tubes. At the speed commonly used to produce L-PRF (2700 rpm, or 3400 rpm in the case of Salvin), all tested centrifuges (except Intra-Spin) are largely above this threshold of 1 for resonance, and it is now necessary to evaluate in details the effects of these vibrations on the cell content and fibrin architecture of each L-PRF clot produced with these machines **[9]**.

The A-PRF centrifuge (Advanced Platelet-Rich Fibrin) is an interesting case, as it was suggested to be used with a very low speed (1300 rpm) in order to produce a PRF-like clot called A-PRF. This is actually a quite rare approach, as a too low speed does not allow a good separation of the blood components and the activation of leukocytes. Moreover, the vibrations of this machine at this low speed are already above the threshold of 1 marking the theoretical limit of resonance. In theory, the best configuration for L-PRF would be to have a reasonable speed (around 2700 rpm) for adequate blood separation, and no vibration or resonance to protect the cell content **[9]**, as the adequate collection of the leukocyte appeared as an importance parameter for the clinical effect of these technologies **[32,33]**. Therefore, A-PRF could serve as an interesting example to compare with the original L-PRF, and to illustrate the impact of speed and vibrations on the final aspect and content of a PRF clot.

Finally, this study raises very serious concerns about the whole PRP literature. PRP centrifuges are in general a bit larger and heavier than PRF centrifuges, and should therefore in theory present a lower risk of vibrations (they seem often to vibrate less when a hand is placed on it). However, the centrifuges tested in this study are also used to produce PRP through another protocol, and the inadequate conception of larger centrifuges can lead to the same risk of vibrations integrated to the level of resonance, whatever their size and weight. Moreover, many PRP methods are also using much higher centrifugation speeds and sometimes g forces **[2,3]** than the L-PRF method (often considered to be a very soft method), as they are supposed to make a very sharp separation of the blood components. For all these reasons, it would be interesting to evaluate more seriously the vibrations of all centrifuges available for PRP on the market, in order to evaluate if this has an impact on the final cell content of the PRP and its biological effect.

5. Conclusion

This article is the first study analyzing the intrinsic differences between 4 PRP/PRF centrifuges available on the market. Using the centrifuges in the same conditions and at the same rotational speed, large discrepancy in vibration levels appeared from one machine to another, and 3 of them reach quickly a threshold of resonance. We can therefore extrapolate that if the protocols were identical, the only variable was the vibration characteristics of the different centrifuges. By far, the most stable machine tested is the original machine (now termed Intra-Spin) used since the early development of the L-PRF technology. At the classical speed of production of L-PRF, the level of undesirable vibration on this centrifuge is between 4.5 and 6 times lower than with other centrifuges. Moreover, Intra-Spin always remains under the threshold of resonance, unlike the 3 other tested machines. It is now necessary to evaluate the impact of the vibration parameter on the architecture and cell content of the L-PRF clots produced with these 4 different machines.

Disclosure of interests

The authors have no conflict of interest to report.

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Author Contributions

All authors participated to the technical design and organization of the study, the treatment of data and to the elaboration of the manuscript. DDE, NP, BSK and MDC were in charge of the collection of the materials and raw data.

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