applied sciences

MDPI

Article

A Practical Guide to Class IIa Medical Device Development

Adél Hinsenkamp [1,*], Dorottya Kardos [1], Zsombor Lacza [2,3] and István Hornyák [1,2]

1 Institute of Translational Medicine, Semmelweis University, 1094 Budapest, Hungary; dorottya.kardos@orthosera.com (D.K.); istvan.hornyak@orthosera.com (I.H.)
2 Orthosera GmbH, 3500 Krems an der Donau, Austria; zsombor.lacza@orthosera.com
3 Institute for Sports and Health Sciences, University of Physical Education, 1123 Budapest, Hungary
* Correspondence: hinsenkamp.adel-gyongyver@med.semmelweis-univ.hu

Received: 23 April 2020; Accepted: 20 May 2020; Published: 24 May 2020

Abstract: There are many beneficial medical device ideas based on clinical needs and laboratory research, but medical device development is an expensive, time-consuming and complex challenge. Research and quality management, which are both needed to develop a medical device, are two distinct fields, initiated by a researcher or a clinician having a concept for a medical device, and it is often challenging to find and achieve the proper steps to create a licensed product. Thus, in this paper, we demonstrate the required mindset and main steps of the medical device development procedure through an existing example, a Class IIa medical device, called hypACT Inject Auto. HypACT is a specific syringe, which is capable of blood drawing and serum from platelet-rich fibrin (SPRF) isolation in one step in a closed system. SPRF is intended to be used to improve joint functions in the case of musculoskeletal diseases, specifically osteoarthritis.

Keywords: medical device development; practical guide; serum from platelet rich fibrin

1. Introduction

Musculoskeletal diseases including rheumatoid arthritis and osteoarthritis are a leading cause of functional decline and loss in quality of life [1]. Changes in lifestyles in the Western world population including increasing obesity and inactivity are putting populations at high risk of developing these conditions [2]. Specifically, osteoarthritis of the knee is projected to increase exponentially in the coming years; it is estimated that a tenth of the population aged over 50 years will be affected [3]. The direct cost of primary knee replacement is increasing, as well as the indirect costs, such as disability, rehabilitation and complications. The typical duration of the treatment gap (until surgical intervention is inevitable) in patients who undergo arthroplasty is 10 years [4]. However, using autologous blood derivatives can delay the need for total knee replacement surgery, and improve the quality of life by decelerating the progression of arthritis and ameliorating tissue repair in the tissues of the knee [5,6].

Human blood derivatives or blood products are fractions of human blood which have therapeutic uses, including red blood cell and thrombocyte concentrates, human serum albumin, coagulation factors, serum products, and immunoglobulin concentrates and other plasma products isolated both from whole blood or by apheresis. Plasma products are produced using anticoagulants, most commonly citrate or Ethylenediaminetetraacetic acid (EDTA). Serum products undergo the process of blood clotting, thus it contains activated platelets, while in plasma products, additional platelet activation may be needed, generally using thrombin.

Blood derivatives are also widely used in laboratory experiments and cell therapies as medium supplementation as many cell cultures require a source of growth factors, hormones, vitamins, transport proteins and trace elements for their growth; this source is most commonly fetal bovine serum (FBS) [7].

However, the use of this non-human serum has some disadvantages in terms of clinical applicability, reproducibility and animal welfare concerns. There are many alternatives to FBS among human blood derivatives like human serum albumin (HSA) [8], serum [9], platelet-rich plasma [10], and serum from platelet-rich fibrin (SPRF) [11–13], which can evade the drawbacks of FBS and may have an even higher proliferative effect.

Plasma products, especially platelet rich plasma (PRP), are in widespread use in tissue regenerative and reparative applications, such as in scar management [14], and orthopaedics, in musculoskeletal disorders like joint diseases or osteonecrosis [15,16]. The aim of using PRP in joint diseases is to relieve pain and improve the joint function. There are several methods to isolate PRP [17], but the high platelet concentration is common, and thus it is often used by physicians for treating knee and hip osteoarthritis with altering results [6], as the activated platelets release a combination of growth factors and cytokines [18], which induce various responses in the tissues of the joint.

Platelet rich fibrin (PRF) membrane is a serum product which is used most commonly in hard and soft tissue engineering [19–21], such as wound healing [22], and dentistry, in oral, maxillofacial [23,24] and in periodontal plastic surgeries [20,25,26]. PRF is a biodegradable scaffold with noted cell adhesive and proliferative effects promoting revascularization [27,28]. Another advantage of PRF and its derivatives is that they do not require anticoagulants and bovine thrombin during their isolation.

The serum derived from PRF (called SPRF: serum from platelet-rich fibrin, or PRF exudate) is another option for soft and hard tissue regeneration [29], as it was found to have an outstanding cell proliferative effect on human mesenchymal stem cells [30], osteoblasts, chondrocytes and adipocytes [5,11–13]. It can be used in bone grafting [31] and implant surface functionalization [32], in dental applications [33], or treating osteoarthritis [5]. SPRF has slightly different features than PRP, as its growth factor and cytokine milieu are highly natural, because PRF does not contain an extremely large number of platelets and they release their contents over a longer period of time [34], and thus the cytokine level of SPRF is also lower than that of PRP.

The existing methods of SPRF production aim to squeeze out PRF exudate from the PRF clot produced in vacuum blood collection tubes. Blood is drawn into glass tubes or glass containing plastic tubes, then it is immediately centrifuged in one step, but using various centrifugation protocols [35], and whole blood gets separated into the red blood cell containing fraction and fibrin clot, which is formed during centrifugation helped by the activating effect of glass. SPRF can be squeezed out from the fibrin clot. The most common method is placing PRF on a sterile grid and pressing out PRF exudate [32]. There is a medical device, called the PFR box, for the same indication, which contains compression wells to prepare dense PRF cylinders, while the serum exudate is collected in the second level of the box. It is a user-friendly device that allows one to prepare PRF membranes in a sterile and protected environment, while the SPRF squeezed out from the clots is a secondary product [36]. Using a centrifuge or a shaker and a vortex may be another option to separate PRF exudate from PRF [37]. These methods require, besides a centrifuge, blood collection tubes and other equipment to produce sterile SPRF, including a laminar flow hood as the procedures require open handling of the PRF membrane. For clinical applications, where sterile isolation may be problematic, we developed a syringe-shaped closed system for SPRF production.

The HypACT Inject Auto device was designed for orthopaedic surgeons who intend to treat knee osteoarthritis using autologous serum in a point of care therapy. During the procedure, blood is drawn from the patient and SPRF is isolated, which is then injected into the patient's knee immediately, while the sterility of the serum can be maintained. The device is a sterile single-use syringe, designed for blood drawing and SPRF isolation aseptically and simply in a closed system. The principle of SPRF production is the same as used in a stepwise isolation, but the device makes the process easier, faster and safer. It works as a syringe, which contains a glass part to promote blood coagulation and platelet activation. After blood drawing, the device gets immediately centrifuged and PRF is formed inside the syringe. The red blood cell-containing fraction gets into a waste container part and can be discarded. SPRF can be squeezed out from the fibrin matrix using the plunger part of the device, while the PRF clot

becomes a flat membrane inside the device. Attaching a needle onto the end of the device allows the physician to inject the SPRF directly to the knee of the patient. Besides the device, only blood drawing needles and a swing-rotor centrifuge for 50 mL conical centrifuge vials are needed for the treatment.

As medical device development is an expensive, difficult and time-consuming process, in this article we aim to demonstrate the main processes, scheduling and required mindset of medical device development, manufacturing and regulation steps on the actual steps of a Class IIa medical device development. We used this HypACT device to demonstrate the steps of medical device development. We intend to give an impression on the complexity of the processes [38], requirements and human resources that need to work together in order to achieve a medical device product. The demonstrated development was presented from a technological point of view, with the addition of the mandatory regulatory and quality management (QM)-related requirements in the realization of an actual medical device that was put on the market.

2. The Steps of Medical Device Development in General

2.1. Technological Steps

1. At first, the intended use of the medical device must be defined. The intended use is the primary use purpose of the product, including medical indication and patient group.
2. Next, the functional requirement is formulated. The requirements will form the basis for the development of the system and be used to verify and validate the design changes. These requirements need to be translated to functional requirement specifications, which will become design inputs for manufacturing.
3. The design input is the base of the device planning and engineering—it means that the actual parameters regarding operation (usage, risk, standards) and physical appearance of the device are created. The parameters needed for implementation, such as the characteristics and dimensions of the product, are collected as functional requirement specification (Table 1). The design output consists of drawings, specifications and manufacturing instructions—it describes all parts of the device. These will become part of the design and development documentation of the device and therefore also part of the technical documentation.
4. Prototypes are prepared according to the design output and the approved prototype can go into small quantity production. The produced devices are to be used mainly for process validation. Sterilisation and packaging plans must be finalized, and then these need to be validated as well. The design output is suggested to be patented if the medical device is novel, inventive and industrially applicable.

Table 1. Functional requirement specification.

Technical Requirements	Characteristics and Numerical Specifications (Design Input)
Use	Single use
Sterilisation	Ethylene oxide
Materials	The device must conform to ISO 10993-1 as per the essential requirement of 93/42/EEC.
Volume Ratio	12 mL whole blood can be drawn The ratio of the top and bottom parts must be 40% top (serum fraction) and 60% bottom (waste). Note that 4.8 mL serum will be in the glass chamber after the centrifuging procedure
Mass of device	Total weight of the system with all parts, except for the plunger part, plus blood included will be 54 g
Dimensions of the Device	The dimensions of the device, including max diameter, length and conical bottom angle are equivalent to a 50 mL conical centrifuge tube
Packaging	Device packaging is to be suitable for ethylene oxide sterilization
Users	Nurse, Doctor
Medicinal Substance	None
External Interfaces	Swing-out rotor centrifuge for 50 mL conical centrifuge vials

2.2. Quality Management and Regulatory

A quality management system (QMS) is required for medical device certification, preferably according to ISO 13485. Compared to the technological point of view, the QMS focuses on reducing risks and ensuring that the processes are foreseeable and adequately documented, and thus controllable throughout the complete lifecycle of the device.

The quality management and regulatory steps are the following:

1. Complying risks and hazards and their management according to ISO 14971.
2. Classification of the device according to the applicable medical device regulations (MDR), based on the markets that are planned to be accessed. In our example, the EU is the main focus. The classification is based on the risks relating to the device—there are three main classes, Class I, Class II and Class III. This determines if the device needs to be sterile, and gives a basic idea on the magnitude of the costs and complexity of the device.
3. The essential requirements (general safety and performance according to the new MDR) of the devices and all the processes that are in connection with the device are regulated by standards and it is required to be gathered according to the applicable medical device directives (MDD) and regulations (Table 2). Essential requirements are divided into two parts—Part I: general requirements and Part II: requirements for design and construction. A checklist can be obtained through the Official Journal of the European Union to identify the applicable standards. All the process validations need to be performed that are required in the applicable standards and regulations after the design and development phase is over.
4. Biological evaluation needs to be performed according to ISO 10993-1 to ensure from biological and toxicological aspects, and that the use of the device is safe for both the patient and the users (Table 3).
5. Accomplishing clinical evaluation. Clinical evaluation is either the analysis and demonstration of equivalence [39] using publicly available clinical data regarding the clinical, technical and biological characteristics of the medical device compared to relevant medical devices on the market, or performing a clinical investigation [40] to determine its clinical safety and effectiveness, that is stated in the intended use.

Table 2. Essential requirement checklist.

Standard	Regarding Subject
ISO 13485:2016	Medical Devices—Quality Management Systems—Requirements for Regulatory Purposes
ISO 14971:2007	Medical devices—Application of risk management to medical devices
ISO 11607-1:2019	Packaging for terminally sterilised medical devices—Part 1: Requirements for materials, sterile barrier system and packaging systems
ISO 11607-2:2019	Packaging for terminally sterilised medical devices—Part 2: Validation requirements for forming, sealing and assembly processes
EN 868-5:2018	Packaging for terminally sterilised medical devices—Part 5: Sealable pouches and reels of porous material, and plastic film construction—Requirements and test methods
EN 556-1:2002	Sterilisation of medical devices—Requirements for medical devices to be designated STERILE- Part 1: Requirements for terminally sterilised medical devices
EN ISO 80369-7:2016	Conical fittings with a 6% (Luer) taper for syringes, needles and certain other medical equipment—Part 1: General requirements
EN ISO 80369-7:2016	Conical fittings with a 6% (Luer) taper for syringes, needles and certain other medical equipment—Lock fittings
ISO 10993-1:2018	Biological evaluation of medical devices—Part 1: Evaluation and testing within a risk management process

Table 2. *Cont.*

Standard	Regarding Subject
ISO 10993-4:2017	Biological evaluation of medical devices—Part 4: Selection of tests for interactions with blood
ISO 10993-5:2009	Tests for in vitro cytotoxicity
ISO 10993-9:2009	Framework for identification and quantification of potential degradation products
ISO 10993-10:2010	Tests for irritation and skin sensitisation
ISO 10993-11:2017	Tests for system toxicity
ISO 10993-13:2010	Identification and quantification of degradation products for polymeric medical devices
ISO 1135-4:2015	Transfusion equipment for medical use—Part 4: Transfusion Sets for Single Use
EN 1041:2008+A1:2013	Information supplied by the manufacturer of Medical Devices
ISO 15223-1:2016	Medical devices—Symbols to be used with medical device labels, labelling and information to be supplied—Part 1: General requirements
EN 1422:2014	Sterilisers for medical purposes—Ethylene oxide sterilisers — Requirements and test methods
ISO 7886-1:2017	Sterile hypodermic syringes for single use—Part 1: Syringes for manual use
ISO 7886-4:2018	Sterile hypodermic syringes for single use—Part 4: Syringes with re-use prevention feature
ISO 11135:2014	Sterilisation of health care products—Ethylene oxide—Part 1: Requirements for development, validation and routine control of a sterilisation process for medical devices
ISO 11138-2:2017	Sterilisation of health care products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilisation processes
ISO 11737-1:2018	Sterilisation of medical devices—Microbiological methods—Part 1: Determination of a population of microorganisms on products
ISO 11737-2:2009	Sterilisation of medical devices—Microbiological methods—Part 2: Tests for sterility performed in the definition, validation and maintenance of a sterilisation process
ISO 14937:2009	Sterilisation of health care products—General requirements for characterisation of a sterilisation agent and the development, validation and routine control of a sterilisation process for medical devices
ISO 2233:2000	Packaging—Complete, filled transport packages and unit loads—Conditioning for testing
ISO 14155:2011	Clinical investigation of medical devices for human subjects—Good clinical practice

3. Demonstration of the Steps Based on the hypACT Inject Auto Device

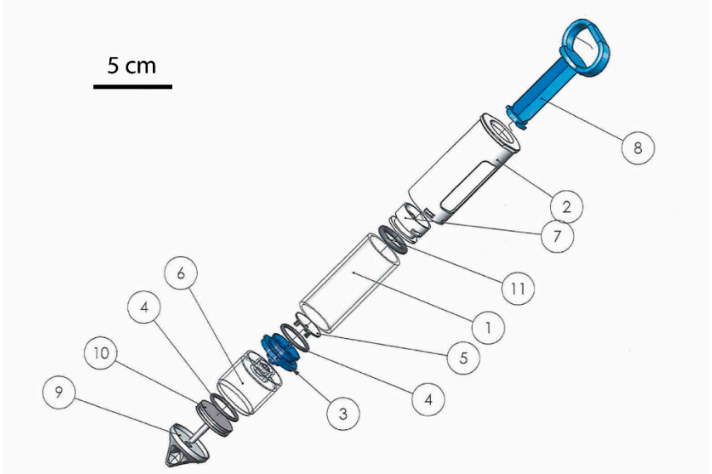
3.1. Technological Steps

1. The intended use of the medical device: The device was developed to accomplish point of care therapy for knee osteoarthritis. It is suitable to draw blood from a patient, and after a physical separation, a concentrated growth factor containing autologous serum (serum from platelet-rich fibrin, SPRF) is isolated aseptically in a closed system. In cases when the physician intends to use SPRF, this serum fraction can be isolated in an aseptic way by the device and injected to the knee of the patient immediately.
2. The user needs were collected and used to create the functional requirement specifications. The user needs were found to be the following:

    - Device can be used in medical facilities.
    - The device can be used in autologous therapy.
    - The plunger can be pulled by one or two fingers.
    - The device has to withstand drawing and injecting therapeutically useful amounts of blood.
    - The clotted serum fraction can be separated from the red blood cells.
    - The device has to fit in a centrifuge bucket where a 50 mL conical centrifuge tube fits.
    - Needles or butterfly needles can be attached to the syringe.
    - The device shall not leak.

- The device shall be used to draw blood from human patients and the contents of the syringe are compatible to be used therapeutically.
- The device can be stored prior to use without any changes occurring.
- Can be centrifuged at high speed for a short period of time.
- Liquid from the clotted serum shall be pressed out.
- No infection occurs when using the device.

3. Design input and output The user needs were converted to functional requirements (Table 1). These were translated to design inputs based on physical parameters, essential requirements and risks. Visualization of the ideas and intensive and constructive communication were conducted between the in-house or contractual developer and the initiator by the fabrication of desk models and prototypes, which resulted in the design outputs. An example to demonstrate a part of the final version of the design outputs of the device can be seen in Figure 1. To simplify the regulatory process, each part of the device is made of approved medical device grade materials.



Item number	Description	Material
1	Glass Cylinder	Bolorosilicate Glass
2	Casing	Polypropylene, Pigment
3	Distal Connector	Polyamide, Pigments
4	O-Ring	Ethylene-propylene-diene rubber
5	Spacer	Polypropylene, Pigment
6	Waste Container	Polycarbonate
7	Syringe Piston	Polypropylene, Pigment
8	Syringe Plunger	Polyamide, Pigments
9	Container Cap	Polyamide, Pigments
10	Waste Piston	Polyamide, Pigments
11	O-Ring	Ethylene-propylene-diene rubber

Figure 1. Design output demonstration, "exploded" visualization of the parts and applied materials of the device.

4. Review and Validation The development process ends with the fabrication of a prototype that was prepared according to the finalized design output, and is accepted by the legal manufacturer. This is followed by a mandatory design review and small quantity production (300–1000 units) can begin for process and manufacturing validations, which were determined by the applicable standards and regulations, e.g., as the above-mentioned biological evaluation. Besides the manufacturing related processes, that need to be validated, two processes need special attention—these are sterilisation and packaging. In the present case, the device was sterilised using ethylene-oxide, due to the glass constituent, which would become amber coloured if gamma radiation was used, thus the sterilisation method was validated according to EN 1422:2014, as listed in Table 2. It is

important to note that not all medical devices need to be sterilised—this requirement needs to be evaluated according to the classification of the device. The packaging of the device consists of a tray (as seen in Figure 2, made of Poly (ethylene terephthalateco-1,4-cylclohexylenedimethylene terephthalate (PETg)) and a Tyvek lid (Tyvek 1073B), and the packaging was tested according to the applicable standards. Packaging validation needs to be performed with special care, as packaging needs to be reliable, being the first and most important barrier throughout the whole lifecycle of the device to ensure sterility from the end of manufacturing during transportation and storage until the device is used by the end user.

The packaging, labelling and instructions for use need to be created according to the applicable standards and are considered as parts of the device; thus, after the medical device design is finalized, then the whole packaged device needs to pass the technology-related process validations. Therefore, the description of the device, also called as the instructions for use (IFU) needs to be part of the design and has to be placed within the packaging before the validation begins. As an example, the main part of the IFU of the present device can be seen in Figure 2. The design of the present device was patented in the EU; the identification number of the granted patent is EP3383269B1.

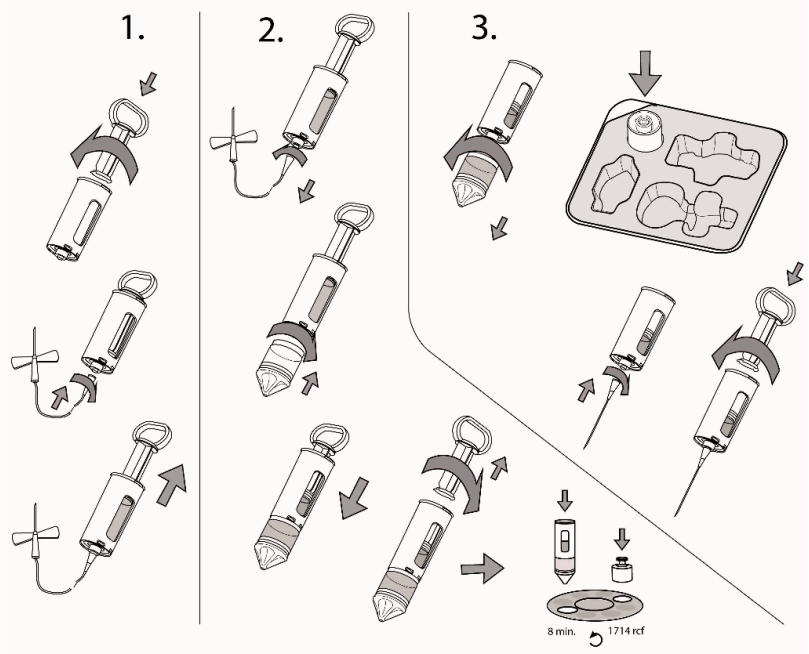


Figure 2. The user guide for the device. (1) Attach the plunger to the piston by rotating it clockwise and attach the butterfly needle. Place the needle in the vein and draw approximately 12 mL venous blood by pulling the plunger slowly. (2) Remove the needle from the vein and detach the needle from the syringe. Attach the waste container to the syringe and use the plunger to push down of the whole blood manually until the waste container is filled. About 60% of the whole blood is enough to fill the waste container. The whole system weighs now approximately 54 g. Remove the plunger by rotating anticlockwise and pulling it. Centrifuge the syringe for 8 min at 3000 rpm. Platelet rich fibrin (PRF) is formed in the syringe part. (3) Detach the waste container and serum from platelet rich fibrin (SPRF) can be pressed out from the syringe using the plunger; approximately 4.8 mL SPRF can be isolated using one device.

3.2. Demonstration of Quality Management and Regulatory Steps and Path

Research and development have to be conducted from different aspects as usual. A quality management system (QMS) is required; it is recommended to write it according to ISO 13485. The QMS has to be prepared parallel with technical documentation. The device must be developed

and manufactured in accordance with the requirements of all applicable standards and regulations. The main aspects are listed below:

1. Risks and hazards and their management need to be identified and the probability of occurrence needs to be reduced to as low as possible according to ISO 14971, Annex E of EN.
2. Classification of the device: in our case, the device was classified according to Annex IX of 93/42/EEC (MDD): The device was categorized as a Class IIa device using rule 3 of the directive. Rule 3: "All non-invasive devices intended for modifying the biological or chemical composition of blood, other body liquids or other liquids intended for infusion into the body are in Class IIb, unless the treatment consists of filtration, centrifugation or exchanges of gas, heat, in which case they are in Class IIa".
3. The essential requirement checklist we used for our device according to MDD can be seen in Table 2.
4. Biological evaluation Biological evaluation was accomplished with the use of Table 3. according to ISO 10993-1. The required tests, that are needed to assure that the device is safe are listed, are based on the nature of contact and the duration of the contact. In our case, the biological evaluation path is highlighted in green.
5. Clinical evaluation In our case, clinical evaluation was achieved by proving equivalence to a product that is already used. We used manually produced SPRF to compare to hypACT SPRF using mesenchymal stem cells with the exact methods and results which can be seen below.

5.1 Maintaining Mesenchymal Stem Cell (MSC) culture

Cell culture procedures were carried out in a laminar flow tissue culture hood. Bone marrow-derived mesenchymal stem cells (MSCs; ATCC, Manassas, VA, USA) were cultured in T-75 flasks in an incubator at 37 °C, 5% CO_2 and 95% humidity. MSCs were maintained in stem cell medium: Dulbecco's modified Eagle's medium containing 4.5 g/L glucose and L-glutamine (Lonza, Basel, Switzerland) supplemented with 10% foetal bovine serum (FBS; EuroClone, Pero, Italy), 1% Penicillin-Streptomycin (Sigma-Aldrich, St. Louis, MO, USA) and 0.75 ng/mL basic fibroblast growth factor (Sigma-Aldrich, St. Louis, MO, USA). The culture medium was refreshed three times a week.

5.2 Preparation of different supplementation containing media

As positive control stem cell medium (FBS medium), as negative controls serum-free medium and human serum albumin (Behring, King of Prussia, Pennsylvania, USA)-containing medium (HSA medium) were used. Phlebotomy occurred under ethical approval (IRB approval number: 33106-1/2016/EKU, 12.07.2016) from healthy donors, men and women, aged 24–45 years. SPRF was isolated using the device as shown in Figure 2 (device SPRF). The device SPRF contains a small amount of red blood cells, which may be harmful to cell viability. They can be eliminated by another centrifugation at 1700 g for 8 min after isolation (twice centrifuged device SPRF). The reference SPRF was isolated manually. Fifty millilitres of blood was drawn using an ordinary syringe and the blood was poured into a centrifuge tube, containing 10 g glass beads as glass surface to promote blood clotting. It was immediately centrifuged at 1700 g for 8 min, while the fibrin matrix was formed. To squeeze out SPRF from the fibrin matrix, it was centrifuged again at 1700 g. Each cell culture media contained 10% supplementation, except for serum-free and HSA medium, where the amount of HSA was normalized to the protein content of SPRF.

5.3 Comparing the effect of different serum containing media on MSC cell viability

MSCs (4 passages) were seeded onto 24 well plates at a density of 1500 cells/well in 500 µL stem cell medium and the culture medium was changed on the first day to the different supplementation containing media. The medium was changed every two days.

Cell viability was measured on the sixth day using the Cell Proliferation Kit II (XTT; Roche, Mannheim, Germany) according to the manufacturer's instructions. Absorbance was measured by a microplate spectrophotometer (Biotek, Winooski, Vermont, USA) at 480 nm with a reference wavelength at 650 nm. The cells were visualized by Live/Dead staining. MSCs were washed with PBS and stained in PBS, containing 1 µM Calcein-AM (Invitrogen, Carlsbad, CA, USA), 4 µg/mL ethidium homodimer (Invitrogen, Carlsbad, CA, USA) and 20 µg/mL Hoechst (Invitrogen, Carlsbad, CA, USA) for 20 min, then washed again in PBS. Images were taken by an inverse fluorescent Nikon microscope.

5.4 Results

The results of XTT measurement (Figure 3) do not show any significant differences between device SPRF, twice centrifuged device SPRF and glass bead SPRF. One-way analysis of variance (ANOVA) with Tukey's post hoc test was performed to compare the means of groups using Prism 7 software. The significance level was $p < 0.05$ and data are presented as mean ± SEM (n = 4). The results of microscopic imaging show (Figure 4) that MSCs cultured in the three kinds of SPRF media reached roughly similar density in the wells and they have the same MSC-like shape.

5.5 Evaluation

The main conclusion of the clinical evaluation was that the blood separation product prepared by our device is at least as safe to use as a PRF isolation method that is already used in the practice, thus that is a way to claim clinical safety of the device. However, the optimal path to investigate clinical safety of medical devices needs to be evaluated on a case-by-case basis; nevertheless, the present path was applicable for the device. However, due to the requirements of the MDR, clinical equivalence may become more rare and actual clinical investigations will need to be done more often in the future.

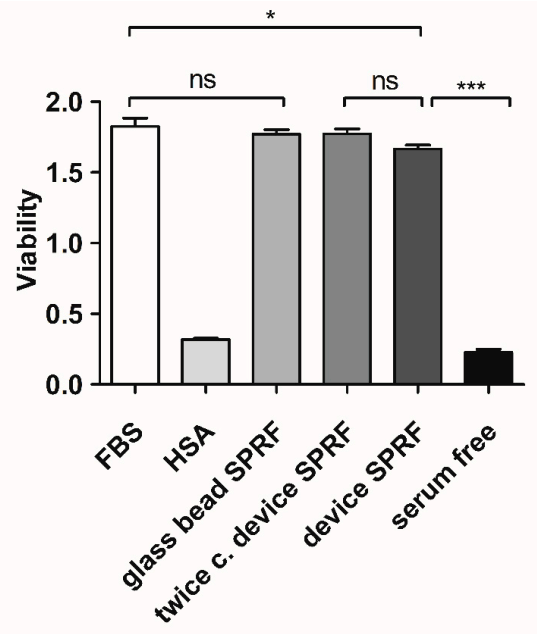


Figure 3. Viability of cells cultured in different serum-containing media. There is no significant difference between SPRF containing media regarding mesenchymal stem cells (MSC) viability. The significance level was $p < 0.05$, where * means that the p-value was between 0.05 and 0.01, and *** means that p-value was lower than 0.001.

Table 3. Medical device categorization path.

Medical Device Categorization by			Endpoints of Biological Evaluation														
Nature of Body Contact	**Contact Duration**		**Physical and/or Chemical Information**	**Cytotoxicity**	**Sensitization**	**Irritation or Intracutaneous Reactivity**	**Material Mediated Pyrogenicity**	**Acute Systemic Toxicity**	**Subacute Toxicity**	**Subchronic Toxicity**	**Chronic Toxicity**	**Implantation Effects**	**Hemocompatibility**	**Genotoxicity**	**Carcinogenicity**	**Reproductive/Develop Mental Toxicity**	**Degradation**
Category	**A-Limited(≤24 h) B-Prolonged (>24 h to 30 d) C-Permanent (>30 d)**																
Surface medical device	Intact skin	A	X	X	X	X											
		B	X	X	X	X											
		C	X	X	X	X											
	Mucosal membrane	A	X	X	X	X											
		B	X	X	X	X		X	X			X					
		C	X	X	X	X		X	X	X	X	X		X			
	Breached or compromised surfaces	A	X	X	X	X	X	X									
		B	X	X	X	X	X	X	X			X					
		C	X	X	X	X	X	X	X	X	X	X		X	X		
Externally communicating medical device	Blood path, indirect	A	X	X	X	X	X	X					X				
		B	X	X	X	X	X	X	X				X				
		C	X	X	X	X	X	X	X	X	X	X	X	X	X		
	Tissue/bone/dentin	A	X	X	X	X	X	X									
		B	X	X	X	X	X	X	X			X		X			
		C	X	X	X	X	X	X	X	X	X	X		X	X		
	Circulating blood	A	X	X	X	X	X	X					X	X			
		B	X	X	X	X	X	X	X			X	X	X			
		C	X	X	X	X	X	X	X	X	X	X	X	X	X		
Implant medical device	Tissue/bone	A	X	X	X	X	X	X									
		B	X	X	X	X	X	X	X			X		X			
		C	X	X	X	X	X	X	X	X	X	X		X	X		
	Blood	A	X	X	X	X	X	X				X	X	X			
		B	X	X	X	X	X	X	X			X	X	X			
		C	X	X	X	X	X	X	X	X	X	X	X	X	X		

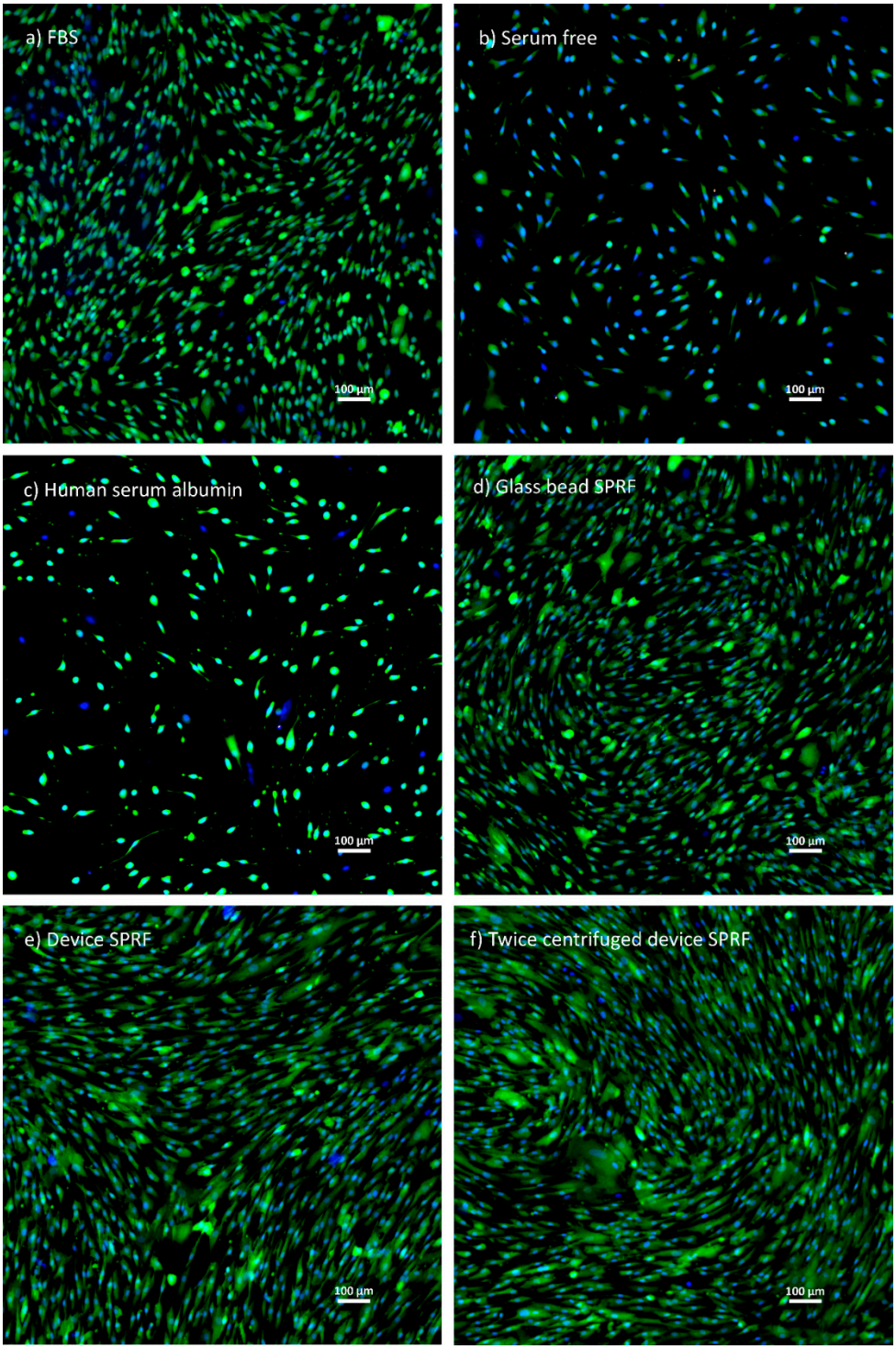


Figure 4. Morphology of MSCs cultured in different serum-containing media. Living cells are green, the nuclei are blue, no dead cells can be seen.

4. Conclusions and Discussion

In summary, the present work aimed to give a basic insight into how to initiate, plan and execute medical device product development. The main conclusion is the importance of planning and proper awareness of the requirements that are needed for medical device development. Before one decides to start a medical device project, careful planning needs to take place, which evaluates the scope and requirements, the timelines of the development and the human resource need, e.g., the knowledge

and skills that are available in-house, which are the ones that need training and which ones need to be outsourced. This article presents the medical device development from a technology-based point of view; however, creating a budget for the whole project is the most important to be realistic. From this viewpoint, the importance of the planning phase becomes easier to understand, as the planning phase costs several hundred thousand euros, whereas the actual development, based on the complexity and safety requirements of the device, is in the million-euro range, from the initial medical device conception until when the product is available on the market.

5. Patents

The device was patented as Apparatus for Preparing Blood Fraction Concentrate by István Hornyák and Zsombor Lacza, the patent number is EP3383269B1.

Author Contributions: Conceptualization, I.H. and A.H.; methodology, I.H., A.H. and D.K.; formal analysis, I.H. and A.H.; investigation, A.H. and I.H.; resources, Z.L.; writing—original draft preparation, A.H. and I.H.; writing—review and editing, I.H. and A.H.; visualization, A.H.; supervision, I.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors are thankful for OrthoSera GmbH for the research support to Semmelweis and Danube Universities and for Arrotek Medical Ltd. Authors Z.L. and I.H. are employees, stock holders, or advisory board members of OrthoSera GmbH, a startup company developing hyperacute serum technology towards clinical applications.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pereira, D.; Ramos, E.; Branco, J. Osteoarthritis. *Acta Med. Port* **2015**, *28*, 99–106. [CrossRef] [PubMed]
2. Woolf, A.D.; Pfleger, B. Burden of major musculoskeletal conditions. *Bull. World Health Organ.* **2003**, *81*, 646–656. [PubMed]
3. Hussain, S.M.; Neilly, D.W.; Baliga, S.; Patil, S.; Meek, R. Knee osteoarthritis: A review of management options. *Scott. Med. J.* **2016**, *61*, 7–16. [CrossRef] [PubMed]
4. London, N.J.; Miller, L.E.; Block, J.E. Clinical and economic consequences of the treatment gap in knee osteoarthritis management. *Med. Hypotheses* **2011**, *76*, 887–892. [CrossRef] [PubMed]
5. Kardos, D.; Marschall, B.; Simon, M.; Hornyak, I.; Hinsenkamp, A.; Kuten, O.; Gyevnar, Z.; Erdelyi, G.; Bardos, T.; Paukovits, T.M.; et al. Investigation of Cytokine Changes in Osteoarthritic Knee Joint Tissues in Response to Hyperacute Serum Treatment. *Cells* **2019**, *8*, 824. [CrossRef] [PubMed]
6. O'Connell, B.; Wragg, N.M.; Wilson, S.L. The use of PRP injections in the management of knee osteoarthritis. *Cell Tissue Res.* **2019**, *376*, 143–152. [CrossRef] [PubMed]
7. van der Valk, J.; Bieback, K.; Buta, C.; Cochrane, B.; Dirks, W.G.; Fu, J.; Hickman, J.J.; Hohensee, C.; Kolar, R.; Liebsch, M.; et al. Fetal Bovine Serum (FBS): Past-Present-Future. *Altex* **2018**, *35*, 99–118. [CrossRef]
8. Nacher, M.; Estil Les, E.; Garcia, A.; Nadal, B.; Pairo, M.; Garcia, C.; Secanella, L.; Novials, A.; Montanya, E. Human Serum Versus Human Serum Albumin Supplementation in Human Islet Pretransplantation Culture: In Vitro and In Vivo Assessment. *Cell Transpl.* **2016**, *25*, 343–352. [CrossRef]
9. Karnieli, O.; Friedner, O.M.; Allickson, J.G.; Zhang, N.; Jung, S.; Fiorentini, D.; Abraham, E.; Eaker, S.S.; Yong, T.K.; Chan, A.; et al. A consensus introduction to serum replacements and serum-free media for cellular therapies. *Cytotherapy* **2017**, *19*, 155–169. [CrossRef]
10. Hosseini, L.; Shirazi, A.; Naderi, M.M.; Shams-Esfandabadi, N.; Borjian Boroujeni, S.; Sarvari, A.; Sadeghnia, S.; Behzadi, B.; Akhondi, M.M. Platelet-rich plasma promotes the development of isolated human primordial and primary follicles to the preantral stage. *Reprod Biomed. Online* **2017**, *35*, 343–350. [CrossRef]
11. Simon, M.; Major, B.; Vacz, G.; Kuten, O.; Hornyak, I.; Hinsenkamp, A.; Kardos, D.; Bago, M.; Cseh, D.; Sarkozi, A.; et al. The Effects of Hyperacute Serum on the Elements of the Human Subchondral Bone Marrow Niche. *Stem Cells Int.* **2018**, *2018*, 4854619. [CrossRef] [PubMed]

12. Vacz, G.; Major, B.; Gaal, D.; Petrik, L.; Horvathy, D.B.; Han, W.; Holczer, T.; Simon, M.; Muir, J.M.; Hornyak, I.; et al. Hyperacute serum has markedly better regenerative efficacy than platelet-rich plasma in a human bone oxygen-glucose deprivation model. *Regen. Med.* **2018**, *13*, 531–543. [CrossRef] [PubMed]
13. Kuten, O.; Simon, M.; Hornyak, I.; De Luna-Preitschopf, A.; Nehrer, S.; Lacza, Z. The Effects of Hyperacute Serum on Adipogenesis and Cell Proliferation of Mesenchymal Stromal Cells. *Tissue Eng. Part A* **2018**, *24*, 1011–1021. [CrossRef] [PubMed]
14. Alser, O.H.; Goutos, I. The evidence behind the use of platelet-rich plasma (PRP) in scar management: A literature review. *Scars Burn Heal* **2018**, *4*. [CrossRef] [PubMed]
15. Fotouhi, A.; Maleki, A.; Dolati, S.; Aghebati-Maleki, A.; Aghebati-Maleki, L. Platelet rich plasma, stromal vascular fraction and autologous conditioned serum in treatment of knee osteoarthritis. *Biomed. Pharm.* **2018**, *104*, 652–660. [CrossRef]
16. Houdek, M.T.; Wyles, C.C.; Collins, M.S.; Howe, B.M.; Terzic, A.; Behfar, A.; Sierra, R.J. Stem Cells Combined With Platelet-rich Plasma Effectively Treat Corticosteroid-induced Osteonecrosis of the Hip: A Prospective Study. *Clin. Orthop. Relat. Res.* **2018**, *476*, 388–397. [CrossRef] [PubMed]
17. Cengiz, I.F.; Oliveira, J.M.; Reis, R.L. PRP Therapy. *Adv. Exp. Med. Biol.* **2018**. [CrossRef]
18. Lang, S.; Loibl, M.; Herrmann, M. Platelet-Rich Plasma in Tissue Engineering: Hype and Hope. *Eur. Surg. Res. Eur. Chir. Forsch. Rech. Chir. Eur.* **2018**, *59*, 265–275. [CrossRef]
19. Kardos, D.; Hornyak, I.; Simon, M.; Hinsenkamp, A.; Marschall, B.; Vardai, R.; Kallay-Menyhard, A.; Pinke, B.; Meszaros, L.; Kuten, O.; et al. Biological and Mechanical Properties of Platelet-Rich Fibrin Membranes after Thermal Manipulation and Preparation in a Single-Syringe Closed System. *Int. J. Mol. Sci.* **2018**, *19*, 3433. [CrossRef]
20. Fan, Y.; Perez, K.; Dym, H. Clinical Uses of Platelet-Rich Fibrin in Oral and Maxillofacial Surgery. *Dent Clin. N. Am.* **2020**, *64*, 291–303. [CrossRef]
21. McDermott, I.D. Patellar chondral defect treatment with a cell-free polyglycolic acid-hyaluronan-based implant and platelet-rich fibrin glue after previously failed microfracture. *SAGE Open Med. Case Rep.* **2019**, *12*. [CrossRef] [PubMed]
22. Sousa, F.; Machado, V.; Botelho, J.; Proenca, L.; Mendes, J.J.; Alves, R. Effect of A-PRF Application on Palatal Wound Healing after Free Gingival Graft Harvesting: A Prospective Randomized Study. *Eur. J. Dent.* **2020**, *14*, 63–69. [CrossRef] [PubMed]
23. Choukroun, J.; Diss, A.; Simonpieri, A.; Girard, M.O.; Schoeffler, C.; Dohan, S.L.; Dohan, A.J.; Mouhyi, J.; Dohan, D.M. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part V: Histologic evaluations of PRF effects on bone allograft maturation in sinus lift. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2006**, *101*, 299–303. [CrossRef] [PubMed]
24. Choukroun, J.; Diss, A.; Simonpieri, A.; Girard, M.O.; Schoeffler, C.; Dohan, S.L.; Dohan, A.J.; Mouhyi, J.; Dohan, D.M. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2006**, *101*, 011. [CrossRef] [PubMed]
25. Jung, M.; Lee, J.; Wadhwa, P.; Jiang, H.; Jang, H.-S.; Lee, E. Bone Regeneration in Peri-Implant Defect Using Autogenous Tooth Biomaterial Enriched with Platelet-Rich Fibrin in Animal Model. *Appl. Sci.* **2020**, *10*, 1939. [CrossRef]
26. Castro, A.B.; Meschi, N.; Temmerman, A.; Pinto, N.; Lambrechts, P.; Teughels, W.; Quirynen, M. Regenerative potential of leucocyte- and platelet-rich fibrin. Part A: Intra-bony defects, furcation defects and periodontal plastic surgery. A systematic review and meta-analysis. *J. Clin. Periodontol.* **2017**, *44*, 67–82. [CrossRef]
27. Miron, R.J.; Zucchelli, G.; Pikos, M.A.; Salama, M.; Lee, S.; Guillemette, V.; Fujioka-Kobayashi, M.; Bishara, M.; Zhang, Y.; Wang, H.L.; et al. Use of platelet-rich fibrin in regenerative dentistry: A systematic review. *Clin. Oral Investig.* **2017**, *21*, 1913–1927. [CrossRef]
28. Meza, G.; Urrejola, D.; Saint Jean, N.; Inostroza, C.; Lopez, V.; Khoury, M.; Brizuela, C. Personalized Cell Therapy for Pulpitis Using Autologous Dental Pulp Stem Cells and Leukocyte Platelet-rich Fibrin: A Case Report. *J. Endod.* **2019**, *45*, 144–149. [CrossRef]
29. Chien, C.S.; Ho, H.O.; Liang, Y.C.; Ko, P.H.; Sheu, M.T.; Chen, C.H. Incorporation of exudates of human platelet-rich fibrin gel in biodegradable fibrin scaffolds for tissue engineering of cartilage. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2012**, *100*, 948–955. [CrossRef]
30. Koh, Y.-G.; Lee, J.-A.; Lee, H.-Y.; Kim, H.-J.; Kang, K.-T. Biomechanical Evaluation of the Effect of Mesenchymal Stem Cells on Cartilage Regeneration in Knee Joint Osteoarthritis. *Appl. Sci.* **2019**, *9*, 1868. [CrossRef]

31. Witek, L.; Tian, H.; Tovar, N.; Torroni, A.; Neiva, R.; Gil, L.F.; Coelho, P.G. The effect of platelet-rich fibrin exudate addition to porous poly(lactic-co-glycolic acid) scaffold in bone healing: An in vivo study. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2020**, *108*, 1304–1310. [CrossRef] [PubMed]
32. Lollobrigida, M.; Maritato, M.; Bozzuto, G.; Formisano, G.; Molinari, A.; De Biase, A. Biomimetic Implant Surface Functionalization with Liquid L-PRF Products: In Vitro Study. *BioMed. Res. Int.* **2018**, *2018*, 9031435. [CrossRef] [PubMed]
33. Li, X.; Yang, H.; Zhang, Z.; Yan, Z.; Lv, H.; Zhang, Y.; Wu, B. Plateletrich fibrin exudate promotes the proliferation and osteogenic differentiation of human periodontal ligament cells in vitro. *Mol. Med. Rep.* **2018**, *18*, 4477–4485. [CrossRef] [PubMed]
34. Kardos, D.; Simon, M.; Vacz, G.; Hinsenkamp, A.; Holczer, T.; Cseh, D.; Sarkozi, A.; Szenthe, K.; Banati, F.; Szathmary, S.; et al. The Composition of Hyperacute Serum and Platelet-Rich Plasma Is Markedly Different despite the Similar Production Method. *Int. J. Mol. Sci.* **2019**, *20*, 721. [CrossRef] [PubMed]
35. Miron, R.J.; Fujioka-Kobayashi, M.; Bishara, M.; Zhang, Y.; Hernandez, M.; Choukroun, J. Platelet-Rich Fibrin and Soft Tissue Wound Healing: A Systematic Review. *Tissue Eng. Part B Rev.* **2017**, *23*, 83–99. [CrossRef] [PubMed]
36. Dohan Ehrenfest, D.M. How to optimize the preparation of leukocyte- and platelet-rich fibrin (L-PRF, Choukroun's technique) clots and membranes: Introducing the PRF Box. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2010**, *110*, 275–278. [CrossRef]
37. Su, C.Y.; Kuo, Y.P.; Tseng, Y.H.; Su, C.H.; Burnouf, T. In vitro release of growth factors from platelet-rich fibrin (PRF): A proposal to optimize the clinical applications of PRF. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2009**, *108*, 56–61. [CrossRef]
38. Levy, A.; Coulthard, M.; Harvey, M.; Talbot, S.; Rich, G. Tube Anchor: Development of a small medical device from concept to market. *BMJ Innov.* **2015**, *1*, 151–156. [CrossRef]
39. Wright, D.J.; Potter, J.F.; Clark, A.; Blyth, A.; Maskrey, V.; Mencarelli, G.; Wicks, S.O.; Craig, D.Q.M. Administration of aspirin tablets using a novel gel-based swallowing aid: An open-label randomised controlled cross-over trial. *BMJ Innov.* **2019**, *5*, 113–119. [CrossRef]
40. Al-Halhouli, A.A.; Al-Ghussain, L.; Bouri, S.; Habash, F.; Liu, H.; Zheng, D. Clinical Evaluation of Stretchable and Wearable Inkjet-Printed Strain Gauge Sensor for Respiratory Rate Monitoring at Different Body Postures. *Appl. Sci.* **2020**, *10*, 480. [CrossRef]