Ministry of Education and Science of the Russian Federation
Federal Independent Educational Institution
«NATIONAL RESEARCH TOMSK POLYTECHNIC UNIVERSITY»

Research School of Chemical and Biomedical Technologies
Direction of training 12.04.04 «Biotechnical systems and technologies»

MASTER'S THESIS

Topic of the work

Preparation and study of piezoelectric hybrid polymer materials

Получение и исследование пьезоэлектрических гибридных полимерных материалов

UDC **621.3.002.3:537.226.8:678.5:620.92**

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Tomsk – 2020

Planned program learning outcomes

Код результата	Результат обучения (выпускник должен быть готов)	Требования ФГОС, критериев и/или заинтересованных сторон
Профессиональные компетенции		
P1	Применять глубокие специальные естественнонаучные, математические, социально-экономические и профессиональные знания в инновационной инженерной деятельности при разработке, производстве, исследовании, эксплуатации, обслуживании и ремонте современной биомедицинской и экологической техники	Требования ФГОС (ОК-2, ОПК-2), Критерий 5 АИОР (п. 5.2.1), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P2	Ставить и решать инновационные задачи инженерного анализа и синтеза с использованием специальных знаний, современных аналитических методов и моделей	Требования ФГОС (ОПК-1, 3; ПК- 1 – 4), Критерий 5 АИОР (п. 5.2.2), согласованный с требованиями международных стандартов EUR-ACE и FEANI
P3	Выбирать и использовать необходимое оборудование, инструменты и технологии для ведения инновационной практической инженерной деятельности с учетом экономических, экологических, социальных и иных ограничений	Требования ФГОС (ОК-9, ПК-10, 14, 18). Критерий 5 АИОР (пп. 5.2.3, 5.2.5), согласованный с требованиями международных стандартов EUR-ACE и FEANI
P4	Выполнять комплексные инженерные проекты по разработке высокоэффективной биомедицинской и экологической техники конкурентоспособной на мировом рынке	Требования ФГОС (ОК-2, 3; ПК-5 – 11, 14), Критерий 5 АИОР (пп. 5.2.3, 5.2.5), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P5	Проводить комплексные инженерные исследования, включая поиск необходимой информации, эксперимент, анализ и интерпретацию данных с применением глубоких специальных знаний и современных методов для достижения требуемых результатов в сложных и неопределенных условиях	Требования ФГОС (ОК-2, 3; ОПК-5, ПК-1 – 4). Критерий 5 АИОР (пп. 5.2.2, 5.2.4), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P6	Внедрять, эксплуатировать и обслуживать современное высокотехнологичное оборудование в предметной сфере биотехнических систем и технологий, обеспечивать его высокую эффективность, соблюдать правила охраны здоровья и безопасности труда, выполнять требования по защите окружающей среды	Требования ФГОС (ОПК-1, 2), Критерий 5 АИОР (пп. 5.2.5, 5.2.6), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
Универсальные компетенции		
P7	Использовать глубокие знания в области проектного менеджмента для ведения инновационной инженерной деятельности с учетом юридических аспектов защиты интеллектуальной собственности	Требования ФГОС (ОПК-2; ПК-14, 15). Критерий 5 АИОР (п. 5.3.1), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P8	Владеть иностранным языком на уровне, позволяющем активно осуществлять коммуникации в профессиональной среде и в обществе, разрабатывать документацию, презентовать и защищать результаты инновационной инженерной деятельности	Требования ФГОС (ОК-1), Критерий 5 АИОР (п. 5.3.2), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P9	Эффективно работать индивидуально и в качестве члена и руководителя команды, состоящей из специалистов различных направлений и квалификаций, с делением ответственности и полномочий при решении инновационных инженерных задач	Требования ФГОС (ОК-3, ОПК-3; ПК-3, 12, 13), Критерий 5 АИОР (п. 5.3.3), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P10	Демонстрировать личную ответственность, приверженность и готовность следовать профессиональной этике и нормам ведения инновационной инженерной деятельности	Критерий 5 АИОР (п. 5.3.4), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P11	Демонстрировать глубокие знание правовых социальных, экологических и культурных аспектов инновационной инженерной деятельности, компетентность в вопросах охраны здоровья и безопасности жизнедеятельности	Критерий 5 АИОР (п. 5.3.5), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*
P12	Самостоятельно учиться и непрерывно повышать квалификацию в течение всего периода профессиональной деятельности	Требования ФГОС (ОК-2, 4; ОПК-4), Критерий 5 АИОР (п.5.3.6), согласованный с требованиями международных стандартов *EUR-ACE* и *FEANI*

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APPROVED BY
Head of the Program
_____ F.A. Gubarev
_____ 09.03.2020

ASSIGNMENT
for the Master's Thesis completion

In the form:

Master's Thesis

For a student:

Group	**Full Name**
9DM8I	Andimadam Madana Saravanan Jekhan

Topic of the work:

Preparation and study of piezoelectric hybrid polymer materials **Получение и исследование пьезоэлектрических гибридных полимерных материалов**	
Approved by the order of the Head (date, number)	

Deadline for completion of the Master's Thesis:	

TERMS OF REFERENCE:

Initial data for work: *(the name of the object of research or design; performance or load; mode of operation (continuous, periodic, cyclic, etc.); type of raw material or material of the product; requirements for the product, product or process; special requirements to the features of the operation of the object or product in terms of operational safety, environmental impact, energy costs; economic analysis, etc.).*	Fabrication of PLLA-GO/rGO scaffolds via electrospinning To perform to a comprehensive study of GO/rGO impact on the morphology, crystalline and molecular structures of biodegradable PLLA-based scaffolds The elaborated of PLLA-GO/rGO biocomposites scaffolds has a great potential for regenerative medicine.
List of the issues to be investigated, designed and developed *(analytical review of literary sources in order to elucidate the achievements of world science and technology in the field under consideration, the formulation of the problem of research, design, construction, the content of the procedure of the research, design, construction, discussion of the performed work results, the name of additional sections to be developed; work conclusion).*	1) Literature review; 2) obtaining the homogeneous and defects free PLLA-GO/rGO samples 3)comprehensive analysis of GO/rGO impact on crystalline structures of biodegradable PLLA-based scaffolds 4) Financial management, resource efficiency and resource conservation; 5) Social responsibility; 6) Conclusion.
List of graphic material *(with an exact indication of mandatory drawings)*	Fabrication schematic diagram Detail drawing List of items
Advisors on the sections of the Master's Thesis	
Chapter	**Advisor**
Section «Financial Management, Resource	Senior Lecturer, Department of Social and

Efficiency and Resource Saving»	Human Sciences Potekhina Nina Vasilyevna
Section «Social Responsibility»	Associate Professor of General Technical Disciplines Mikhail Vladimirovich Gorbenko

Date of issuance of the assignment for Master's Thesis completion according to a line schedule	

The task was issued by the Scientific Supervisor and Technical Advisor:

Position	Full Name	Academic degree, rank	Signature	Date
Associate Professor	Dr. Roman A. Surmenev	PhD		

The assignment was accepted for execution by the student:

Group	Full Name	Подпись	Дата
9DM8I	Andimadam Madana Saravanan Jekhan		

TASK FOR SECTION
"FINANCIAL MANAGEMENT, RESOURCE EFFICIENCY AND RESOURCE SAVING"

To a student:

Group	Full name
9DM8I	Andimadam Madana Saravanan Jekhan

School	Research School of Chemical and Biomedical Technologies	**School Department**	RSCBT
The level of education	Master	**Direction / specialty**	12.04.04 Biotechnical systems and technologies

Background data to the section "Financial management, resource efficiency and resource saving":	
1. Resource cost of scientific and technical research (STR): material and technical, energetic, financial and human	Salary of the head - 49141 rub. Engineer's salary - 17890rubles
2. Expenditure rates and expenditure standards for resources	Electricity costs – 5,8 rub per 1 kW
3. Current tax system, tax rates, charges rates, discounting rates and interest rates	Labor tax – 27,1 %; Overhead costs – 30%;
The list of issues to be investigated, designed and developed:	
1. Assessment of commercial and innovative potential of STR	comparative analysis with other researches in this field;
2. Development of charter for scientific-research project	SWOT-analysis
3. Scheduling of STR management process: structure and timeline, budget, risk management	calculation of working hours for project; creation of the time schedule of the project; calculation of scientific and technical research budget;
4. Resource efficiency	integral indicator of resource efficiency for the developed project.
The list of graphic material (with the exact indication of the mandatory drawings):	
1. Competitiveness analysis *2. SWOT- analysis* *3. Gantt chart and budget of scientific research* *4. Assessment of resource, financial and economic efficiency of STR* *5. Potential risks*	

Date of assignment for the section on a linear schedule	3.02.2020

Assignment issued by a consultant:

Position	Full name	Degree, title	Signature	Date
Associate professor	E.V. Menshikova	Candidate of Philosophy Sciences -		

The task was accepted for execution by the student:

Group	Full name	Signature	Date
9DM8I	A.M.S.Jekhan		

TASK FOR SECTION

"SOCIAL RESPONSIBILITY"

To a student:

Group		**Group**	
9DM8I		A.M.S.Jekhan	
School	Research School of Chemical and Biomedical Technologies	**Department**	RSCBT
The level of education	Master's	**Direction / specialty**	12.04.04 Biotechnical systems and technologies

Background to the section "Social Responsibility":	
1. Characteristics of the object of study (substance, material, device, algorithm, method, working area) and its areas of application	The object of study is to fabricate a biodegradable piezo PLLA-GO/rGO scaffolds via electrospinning, and study its structural and morphology ,influences of GO/rGO nanofillers in the PLLA matrix for regenerative tissue
The list of issues to be investigated, designed and developed:	
1. Legal and organizational security issues:	1. GOST 12.2.032-78 Occupational safety standards system (SSBT). Workplace while doing work while sitting. General ergonomic; 2. The Labor Code of the Russian Federation dated December 30, 2001 N 197; 3. GOST 12.1.038–82 SSBT Electrical safety. Maximum permissible levels of contact voltage and currents 4. SN 2.2.4 / 2.1.8.562–96 Noise at workplaces, in residential public buildings (approved by the Decree of the State Committee for Sanitary and Epidemiological Supervision of the Russian Federation No. 36 October 31, 1996). 5. GOST 12.2.049-80 Occupational safety standards system (SSBT). Industrial equipment. General ergonomic requirements.
2. Industrial safety 2.1. Analysis of the identified harmful factors in the development and operation of the designed solution in the following sequence:	1. The increased gas contamination of the air of the working area; 2. Deviation of microclimate indicators;

2.2. Analysis of the identified hazards in the development and operation of the designed solution in the following sequence:	3. Noise level indicators; 4. Fire; 5. Electrical current
3. Ecological safety:	Environmental pollutions: 1. Household waste; 2. Chemical waste; The atmosphere is not polluted
4. Safety in emergency situations:	1. Electricity 2. Fire safety
Date of assignment for the section on a linear schedule	

Assignment issued by a consultant:

Position	Full name	Degree, rank	Signature	date
Associate professor	M.V. Gorbenko	PhD		

The task was accepted for execution by the student:

Group	Full name	Signature	date
9DM8I	A.M.S.Jekhan		

Abstract

Master’s Thesis contains 95 pages, 22 figures, 7 tables, 103 references, 3 appendix.

Keywords: electrospinning, biopolymer scaffolds, piezoresponse, crystalinity.

The object of the study is biodegradable piezoresponse PLLA–GO/rGO scaffolds.

Objective: Comprehensive study of biodegradable hybrid electrospun Fibers based on piezoelectric poly (L–lactic acid) and GO and rGO nanofillers for regenerative tissue engineering

In the course of the study, Since the structure of solid state of PLLA consists of mainly amorphous phase, PLLA demonstrates a weak piezoelectric and mechanical performance that can limit their successful applications for regenerative bone tissue engineering Therefore, to change the crystalline structure of PLLA, 2D rGO and GO nanofillers have been used. According to the literature analysis, the effect on the structure of the PLLA, allowing to achieve a maximum piezoelectric response of the polymers doping rGO/GO.

As a result of the study, the addition GO/rGO fillers in the 3-D PLLA scaffolds demonstrate the presence of β/α-phase of PLLA. In turn, a clear presence of the α and β-phases in PLLA allows to expect a pronounced piezoelectric response for tension and compression at the same time.

Scope Thus, the elaborated of biocomposites scaffolds has a great potential for regenerative medicine.

Economic efficiency/importance of the work: calculations of the project efficiency showed that the project has a high level of scientific and technical effect, which fully justifies the economic costs of creating the biodegradable piezoelectric scaffolds. The piezoelectric properties of these rGO/GO-PLLA scaffolds will be study in the future.

Abbreviations

TERM – tissue engineering and regenerative medicine

PLLA – poly (L– lactic acid)

PLA – poly (lactic acid)

PHB – polyhydroxybutyrate

PCL – polycaprolactone

GO – graphene oxide

rGO – reduced graphene oxide

RPM – revolution per minute

SEM – scanning electron microscopy

FTIR – Fourier-transform infrared spectroscopy

TGA – thermogravimetric analysis

DSC – differential scanning calorimetry

XRD – X–ray phase analysis

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Chapter 1. Literature review

1.1. Introduction

Humans lose tissues and organs due to congenital defects, trauma and diseases. Globally, millions of people would benefit immensely if tissues and organs can be replaced on demand. Traditionally, transplantation of intact tissues and organs has been the bedrock to replace damaged and diseased parts of the body [1–4]. The advent of TERM appears to make it possible. Tissue engineering combines cells, scaffolds and growth factors to regenerate tissues or replace damaged or diseased tissues, while regenerative medicine combines tissue engineering with other strategie including cell–based therapy, gene therapy and immunomodulation. TERM is a multidisciplinary science and combines basic sciences such as materials science, biomechanics, cell biology and medical sciences to realize functional tissue/organ repair or reconstruction. With the aging of world population trend intensifying, there is a demand of organ replacements. TERM holds the potential to meet the future needs of patients [5–8]. The aim of TERM is to establish a three–dimensional (3D) cell biomaterial complex, which has similar function as a living tissue/organ and may be used to repair or regenerate injured tissue/organ. The basic requirement for the complex is that it can support cell growth, transportation of nutrition and waste, and gas exchange. TERM usually uses the following three strategies [9, 10]. Cell biomaterial complex system, in which cell–seeded biomaterials are implanted into the body to repair and regenerate tissues/organs [11]. Cell systems, such as stem cell transplantation [12] and biomaterial systems, which will be implanted into body and undergo the process of tissue integration. Tissue engineering and regenerative medicine has been proposed and developed for more than 30 years. While several successful attempts in tissue regeneration have been achieved, TERM is still in its infancy and there are many fundamental questions that remain to be answered, including selection of cell sources, development of tissue–specific materials, development of specialized bioreactors, and construction of complex organs. More importantly, the processes and mechanisms of new tissue/organ formed using these tissue engineered materials in vivo, similarity and difference between these processes with nature tissue/organ development, healing and transformation and final

destination of these materials continue to be the critical concerns in this dynamically developing field. Addressing these questions is the key to the effectiveness, stability, and security of the clinical application of tissue–engineered materials [13–17].

1.2. Bone tissue engineering

For the successful tissue engineering application, the understanding and integration of physics, biology, chemistry, material science, engineering, and medicine is essential. In bone tissue engineering, bone cells, extracellular matrix, intercellular communications, growth factors, and cell–matrix interactions are some components to be understood from a biological perspective [18–21]. In addition to this, another important factor is the three–dimensional structure of the bone. Since cells do not grow in a three–dimensional manner in vitro, designing a scaffold that mimic the bone structure is crucial for the cells to grow to form a new tissue in three–dimensional manner in vivo [22, 23].

1.3 Scaffolds

A scaffold is a temporary template for cells to regenerate and restore functional tissues. Scaffolds support and provide a reservoir for nutrients, water, cytokines and growth factors. In vivo, cells use scaffolds as a temporary matrix where they can deposit and proliferate to induce bone in growth, until new bone tissue is fully restored. Scaffolds also act as a template for a vascularization of newly formed tissues [24–27].

1.3.1 Essential properties for a desirable scaffold

Researchers have been devoted to bone tissue engineering where they develop desirable three–dimensional porous scaffolds with tissue–inducing factors [28]. The three–dimensional porous scaffolds need to be designed for the manipulation of osteoblasts functions as well as guide and induce new bone formation. Materials used in scaffolds also need to be osteoconductive for osteoprogenitor cells to migrate and adhere onto the scaffolds, differentiate and form a new bone [29–34]. The desirable scaffolds should be also biodegradable with proper degradation rate so that the scaffolds will eventually be replaced totally by newly formed bone. Porosity is also

important [35]. Scaffolds should have fully interconnected highly porous structure with high surface to volume ratio for cell in growths and vascularization for newly formed tissue [36–40]. A recent study with the non–biodegradable piezoelectric polymer has shown a necessity of the increase of piezoelectric properties for better biomedical applications [41]. Thus, the scaffold in bone tissue engineering should satisfy the requirements: biocompatiblility, osteoconductivity, osteoinductivity, interconnecte porous structure, proper biodegradability, and mechanical strength [42].

1.4 Electrospinning

Electrospinning is a unique approach of utilizing an electrostatic field to generate ultrafine fibers. Electrospun fibers have highporosity, flexibility and surface area to volume ratio and also a simple, straight–forward and cost–effective method to create fibers with a diameter in the range of 3 nm to 10 μm [43]. Electrospinning is a process in which a high electrostatic field (mainly DC voltage) is applied to produce nanofibers with various properties. Polymeric solution or melt that has to be electrospun is forced by means of a syringe pump through a spinneret to form a pendant drop of the polymeric solution at the tip of the capillary tube (spinneret). The tip of the capillary is connected to an electrode and the other end of the electrode is con–nested to a high DC supply. The electrical forces then draw this pendant drop into a hemispherical shape, commonly known as a Taylor cone [44].The bending in stability stretches the jet thousands of times more than its original size, thus experiencing a large amount of plastic deformation and thereby resulting in ultra fine fibers before arriving at the metallic screen (collector). Figure 1 shows the experimental setup of an electrospinning process.

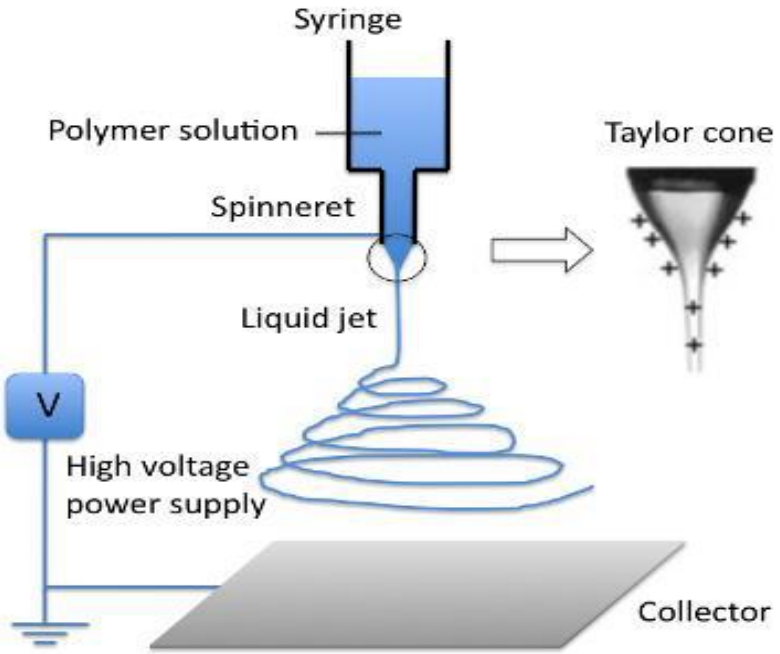


Figure 1 – Electrospinning set up[44]

1.4.1 Electrospinning parameters

Different parameter, which affects the electrospining process, can be split into three categories.

- Solution of the polymer;
- Process conditions (such as applied voltage, distance, feed rate, polarity, needle properties);
- Ambient conditions (such as: temperature, humidity).

Optimization of these conditions leads to product defect–free electrospun nanofibers.

1.4.2 Bioapplications of electrospinning

A variety of synthetic and natural polymers have been used for electrospinning. Biodegradable polymers, such as PLA, PHB, PCL and collagen have been used to produce porous structures for drug delivery and tissue engineering [45]. Drugs, growth factors and cellular components have been added to the solution to incorporate them in the porous matrix for eventual controlled release [46]. This technique can provide several advantages for controlled delivery of drugs, proteins over extended periods to

obtain several investigators have explored the use of electrospun polymer scaffolds for tissue engineering. This novel technique can be used to mimic the architecture of the natural extracellular matrix to produce cell specific scaffolds [47].

1.5 Poly (L–lactic acid)

Biodegradable polymers have gained wide use in biomedical and pharmaceutical industry. A suitable biodegradable polymer would have following properties: (a) metabolization and elimination from the body by normal physiological pathways; (b) easy fabrication; (c) degradation into non toxic substances; (d) no inflammatory reaction after application; (e) degradation product of the biodegradable polymer should be carbon dioxide and water [48–50]. According to these criteria, PLLA and their copolymers are considered as suitable biodegradable polymers. PLA has asymmetric α- carbon which can be described as D–or L–stereo chemical centres (or R or S, respectively) resulting in two enantiomeric forms of poly D–lactic acid (PDLA) and poly L–lactic acid (PLLA) [51]. Figure 2 shows the structure of PLLA. PLLA has melting temperature of approximately 170–180°C and a glass transition temperature (Tg) of approximately 50°C [52]. Due to crystallinity, PLLA is less permeable compared to the amorphous polymers. Crystallinity of PLLA also gives its mechanical strength and stiffness properties. PLLA has been widely studied for drug delivery because of its biodegradability and biocompatibility [53–55]. Molecular weight of the polymer is directly related to polymer chain size, which affects the degradation rate. High molecular weight polymers require more time to degrade. However, for PLLA this effect is opposite because of inverse relation of crystallinity with molecular weight. PLLA can sustain the drug release for a longer period due to its longer degradation half–life [56–58].

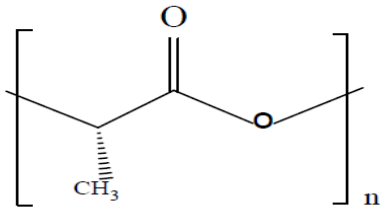


Figure 2– Molecular structure of PLLA

1.5.1 Bioapplication of PLLA

The environmentally friendly qualities of PLLA shown promising results in many biomedical fields such as medical electronics, tissue engineering and regenerative medicine Since the first experiment in 1996 with guinea pigs and rats to study the degradation of PLLA in the body fluids, it has been successfully used for bones fixation and implants [59, 60]. Table 1 summarizes the important biomedical application of PLLA.

Table 1 – Biomedical application of PLLA [12]

Purpose	**Function**	**Examples**
Operation assist	Bonding, closure, separation	Vascular and intestinal anastomosis, bone fixation, wounder cover, hemostasis, vascular embolisation
Damage healing	Scaffold	Wound healing, tissue growth, organ reconstruction
Drug release	Capsulator	Sustained drug release such as antitumor, growth factors to promote healing, antithrombosis, angiogenesis, anti-infection

Thus, PLLA is a wonderful tool in biomedical application because of its biocompatibility, and degradation [60]

.

1.5.2 The structure and piezoelectric effect

One of the key aspects of biological systems is the intricate relationship between the biological response and relevant chemical/physical characteristics, including piezoelectricity, e.g., in amino acid derivatives and calcified tissue. Piezoelectricity has been observed in living tissues including bone dentin and tendon [61–62], among others. It is known that physical exercise, which is associated with an applied stress to the material and translates into a piezoelectric signal from the bone to the living cells, thus helping bone regeneration, although the mechanisms involved bone mineralization and growth are unclear. The piezoelectric activity of the bone was attributed to the collagen and is dependent on the direction of the applied load, frequency, and moisture [63]. Halperin et al. [64] reported that the piezoelectric coefficient for the human bone can go up to 7 pC/N. Biomaterials are a class of natural, synthetic, materials that can interact with biological systems to replace as natural function. These mate rails should be nontoxic non–injurious, and non–immunogenic (i.e., also known as biocompatible) for various applications involving tissue engineering minimally invasive sensors drug delivery etc [65]. Piezoelectric materials are a family of both organic (mostly polymers) and inorganic materials that can convert mechanical force into electricity and vice versa. For example, hair, wool, horns, and hooves are mostly composed of α- keratin with a compactly aligned and polarized α- helical structure. Most parts of the musculoskeletal tissue have a highly collagenous structure. Collagen also has a spiral structure with three helical fibrils. Each collagen fibril shows lateral piezo response along the fibril axis [66–69]. Thus, tissues that are heavily comprised of collagen, including bone, cartilage, ligaments and tendons, are all piezoelectric in nature. Among the many applications for piezoelectric technologies, those that involve interfaces with biological systems represent an exciting area of rapid development. Inorganic piezoelectric materials are biocompatible or can be biocompatible after being encapsulated. This includes materials like lead zircon ate titan ate (PZT) aluminum nitride (AlN), zinc oxide (ZnO), barium titanate ($BaTiO_3$), lithium niobate

(LiNbO3), and quartz. Organic materials, however, are biocompatible and environmentally friendly in nature. The use of PLLA in the medical field has increased due to its biocompatibility, bioresorption, biodegradation, low toxicity, and strong mechanical performance. PLLA is also known for its piezoelectric activity with a piezoelectric constant ranging up to approximately 10 pC/N [13]. It consequently is considered as a promising material for bio applications that can also take advantage of its stress-induced electroactivity, advantageous for bone growth, and regeneration or neural recovery. For many biological and biomedical applications, it is an advantage to process the material in the form of fibers or fiber mats. Membranes and scaffolds of biodegradable polymers find numerous applications in tissue repair and regeneration. The tissue engineering approach relies upon the use of polymer scaffolds, which act as supports for cell adhesion, proliferation, and differentiation, providing them mechanical reinforcement until the regenerated tissue is able to sustain the applied forces “in vivo.” In some cases, scaffolds also help the organization of the produced extracellular matrix. Growth of cells on a polymeric scaffold using the principles of tissue engineering provides a viable “in vitro” model for biological experimentation [16]. Electrospun biodegradable polymer nanofibers are used in tissue engineering scaffold applications for nerve [17], cartilage [18], bone [19], and heart [20] regeneration. Electrospinning allows the production of polymeric nanofibers that closely mimic the fibers in the extra cellular matrix [21] However, when compared to inorganic piezoelectric materials, an organic piezoelectric material often does not have a comparable piezoelectric output. Piezoelectric materials, when processed appropriately, become a powerful biomaterial that can be used for tissue engineering and various biomedical applications such as wound healing due the polarity of piezo electric effect. Thus, PLLA piezo effect plays an important role in the tissue engineering and wound healing process [9, 72]. However, PLLA scaffolds demonstrate weak piezoelectric and mechanical performance that can limit their successful applications as bone scaffolds this is due to its predominant amorphous structure , which limits its crystallinity phase ie, α- and β- crystallinity phases of the

polymer determines its piezoresponse [73].

1.6 Graphene

Graphene is a single layer carbon atom arranged in a closely packed honeycomb of two–dimensional crystalline lattice. Graphene has been investigated extensively due to its unique properties in terms of physical, chemical and mechanical properties [74]. Its electron is in high mobility and the charge carriers in graphene crystals mimic relativistic particles with no rest mass, which is eventually described as massless Dirac fermions. If we think carefully about graphene, it is actually very abundant material since it is a basic building block of natural graphite. The average distance between atoms is about 0.142 nm (1.42 Å) and the spacing between layers (if present) is ~ 0.35 nm [75]. In addition to it, graphene is able to repair its disruptions (holes in the lattice) using carbon atoms which were 'knocked out' from eighbouring edges or by using nearby hydrocarbon contaminations as a building material. This makes graphene a promising candidature and made it very popular and attracted focus of many scientific fields [76].

1.6.1 Graphene oxide

Graphene oxide is often considered as a precursor for graphene production. The biggest difference between graphene and graphene oxide is without doubts presence of various functional groups, which are introduced via strong oxidation of flake graphite. Interestingly, GO was for a long time considered as a graphitic material. It was not until the discovery of single graphene sheet via micromechanical cleavage before the question regarding this material was reexamined and stated that the water dispersion consists of graphene oxide particles [77]. Preparation via GO became very popular mostly because of its high production yield and simplicity oxidation process typically forms epoxy, carboxyl and hydroxyl groups to the graphitic structure, changing the hybridization of reacting carbon atoms from sp2 to sp3. As a result of this transformation the delocalized π system is disrupted and the conductivity is effectively lost. These moieties, which are present out of the planar base then increase the distance between graphitic layers from~0.35 to~0.68 nm in graphite oxide. Increased distance

between layers together with strong hydrophilic and polar character then allows water molecules to penetrate the structure and thus even further increase the distance between layers. Lastly, graphite oxide could be easily dispersed in water and exfoliated using ultrasonication [77]. Figure 3 shows the structure of GO.

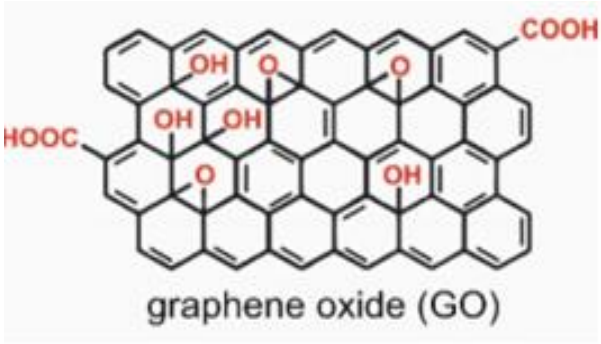


Figure 3 – Molecular structure of graphene oxide

1.6.2 Reduced graphene oxide

Since the first appearance in 2004, rGO contains residual oxygen and other heteroatoms, as well as structural defects. Despite rGO's less than perfect resemblance to pristine graphene, it is still an appealing material that can definitely be sufficient in quality for various applications, but for more attractive pricing and manufacturing processes. Reduced graphene oxide can be used (depending on the specific material's quality) for the same various applications suitable for graphene use, like composite materials, conductive inks sensors and more [74–76]. rGO is often a natural and understandable choice for applications that call for large amounts of material due to the relative ease in creating sufficient quantities of graphene in a relatively low cost. Figure 4 shows the structure of rGO [77].

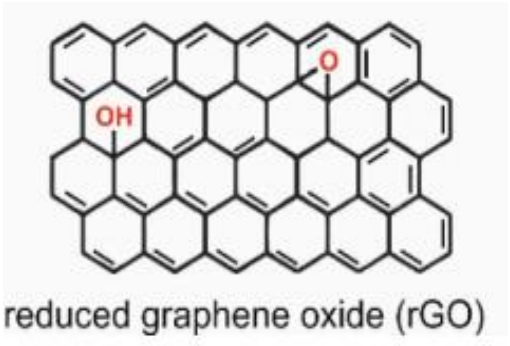


Figure 4 – Molecular structure of reduced graphene oxide

Once reduced graphene oxide has been produced, there are ways to functionalize the material for specific use in different applications. By treating rGO with various chemicals or by creating new compounds by combining rGO with other two – dimensional materials, it's possible to enhance the properties of the compound to suit commercial applications [77].

1.6.3 Influences of GO/rGO fillers in the PLLA matrix

Since the structure of solid state of PLLA consists of mainly amorphous phase, which limits its crystallinity phase ie, α- and β- crystallinity phases of the polymer determines its piezoresponse [3, 5]. PLLA demonstrates a weak piezoelectric and mechanical performance. Moreover, a recent study with the non-biodegradable piezoelectric polymer has shown a necessity of the increase of piezoelectric properties for bioapplications [4, 7, 16]. This disadvantage can be over come by using nanofilleres, such as GO and rGO, to increase its electroactive phase content [6, 8]. GO/rGO attracted great research interest in tissue engineering due to its unique physicochemical, flexibility and biocompatibility. A recent study on PHB–GO/rGO increases piezoresponse of PHB matrix [78]. Therefore, the addition of GO/rGO in the PLLA matrix would enable creating a biodegradable material with the required mechanical and piezoelectric performance for regenerative medicine.

Chapter 2. Experiments

2.1 Materials

Poly (L lactic acid) (PLLA) (Mw~210.000 g/mol), registration number CAS №33135501, PURASORB PL–18, Corbion, Purac Biomaterials Netherland. Reduced graphene oxide (rGO) and graphene oxide (GO), were fabricated at the University of Cologne (Germany) using improved Hummers method. Chloroform, acetone was purchaed from ChP (Ekos–1 JSC Russia).

2.2. Methods

2.2.1 Solution preparation

In a typical preparation process of PLLA–GO composites, for preparing a predetermined volume of polymer solution, it is necessary to calculate the mass of sample as follows (formula 2) [23].

$$m = \frac{V*P*C}{100-C} \quad (2)$$

where m – polymer weight, g; V – volume of solvent, ml; p – density of the solvent, g/cm^3; C – concentration of polymer in solution, %.

2.2.2 Preparation of PLLA–GO/rGO nanocomposites

Different weight concentrations of GO/rGO were dispersed in 4ml of Chloroform and it is placed in ultrasonication (Scientz ID, Ningbo S Cienta Biotechnology Co. Ltd, China) at room temperature for 2 hours. Until GO/rGO particles gets dispersed in chloroform. Dry weight (mass) of PLLA was added in the GO/rGO solution and placed in shaker at room temperature for 3 hours. Until they form a homogenous mixture. At the end, 1 ml of acetone was add to the solution and mixed again. Table 2 shows the detailed weight concentrations of PLLA polymer, graphene oxide; reduce graphene oxide and their corresponding solvents.

Table 2– Electrospinning parameters

PLLA Wt (%)	Feed speed (ml/h)	Distance (cm)	Voltage (kV)
10 /Pure PLLA	0.30	7	5.7
11 / 0.2 % rGO	0.45	6	7.6
10 / 0.2 % rGO	0.60	6	6.9
10 / 0.7 % rGO	0.30	6	4.8
10 / 1.0 % rGO	0.30	8	6.4
11 / 0.2 % GO	0.45	6	7.6
11 / 0.7 % GO	0.45	6	5.6 – 7.0
11 / 1.0 % GO	0.45	6	5.4 – 5.1

2.2.3 Electrospinning

The electrospinning apparatus (developed at Physical Materials Science and Composite Materials Centre National Research Tomsk Polytechnic University, Tomsk, Russia) shown in figure 5.

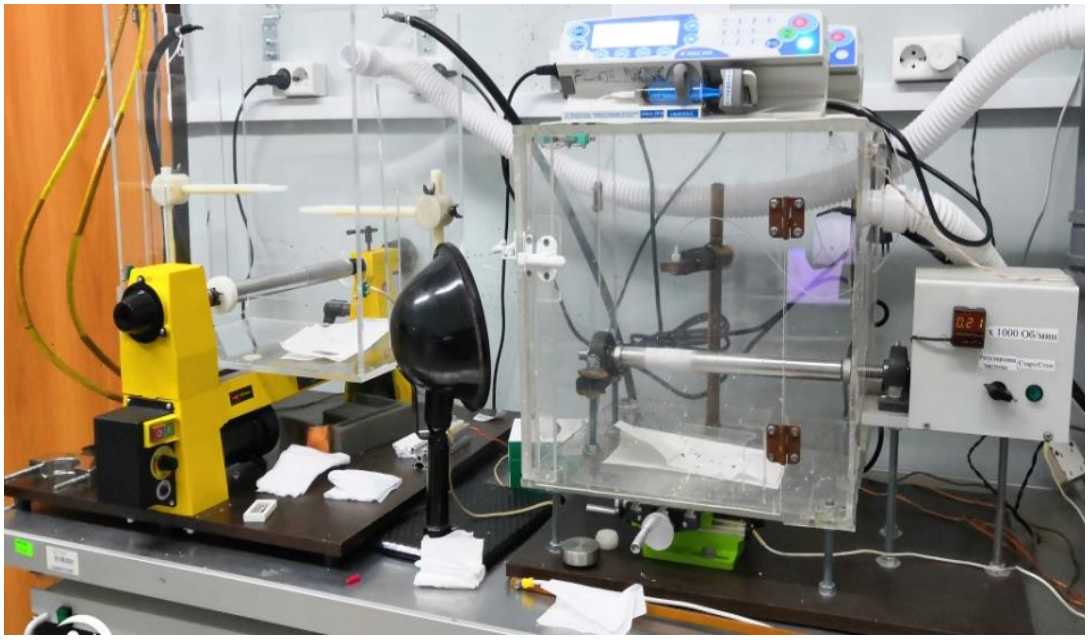


Figure 5 – Electrospinning device

The apparatus consists of pump and syringe with polymer solution (PLLA–GO, PLLA–rGO). The collector and the syringe needle (d=0.51mm/G 21) are subjected

under high DC voltage provided by a power supply (Gamma High Voltage Power Supply ES30P). The syringe needle is attached to the positive output. The syringe with a composite solution is mounted on a pump [Harvard Syringe Pump Model 901]. The samples are collected on a metallic drum which rotates at constant rpm. The experimental values are listed in table 2.

2.2.4 Annealing

PLLA fibrous scaffolds were annealed in a dry oven (Air sterilizer GP 40 SPU, SKTB SPU, Smolensk, Russia) at 160°C for 2 hours and cooled for overnight until room temperature. The annealing parameters (2 hours at 160°C) were chosen from referens [3].

2.2.5 SEM

To study the morphology of the samples, scanning electron microscopy (SEM) was performed using a Quanta 600 electron microscope (Thermo Fisher, Japan) at an accelerating voltage of 10 kV. The diameter of the microfibers was estimated using software Image J, the calculation of the average and standard deviation was performed in Excel.

2.2.6 FTIR

To study the structure of the polylactide matrix, a Nicolet iS10 IR Fourier spectrometer (Thermo Scientific, USA) in the attenuated total reflection mode was used. The spectra were recorded between 4000–525cm^{-1}, with a constant spectral resolution of 4 cm^{-1}. Studies of the effect of GO/rGO additives and annealing on the structure of polylactide matrices were carried out on a Tensor 27 IR Fourier spectrometer (Bruker, Germany) in the attenuated total reflection mode (with ATR attachment, prism–ZnSe), the spectra was recorded between 4000–525cm^{-1}, with a resolution of 4 cm^{-1}. Before each test, a background spectrum was obtained to compensate for the effects of humidity and the presence of carbon dioxide by subtracting the spectra. The studies were carried out at the TPU and TSU Material Science Center for Collective Use, Tomsk.

2.2.7 TGA and DSC

To study the thermal stability of the samples and to study the effect of additives (rGO and GO) on the temperature characteristics of polymer scaffolds, thermogravimetric analysis (TGA) was performed on the thermogravimetric analyzer SDTQ–600SDTQ 600 with mass spectrometer (Thermo Electron Corp). Temperature range up to 1500°C. The sensitivity of the scale is 0.1 μg. The calorimetric accuracy of the air atmosphere is 10°C/min, reproducibility ± 2 %. The sensitivity to DTA is 0.001°C. Samples (weighing about 5 mg) were heated from room temperature to 600°C with a heating rate of 10°C/min in an air stream with a flow rate of 50 ml/min.The equipment (DSC Q2000 V24.10 Build 122, TA Instruments) was used to evaluate the structure of PLLA-GO/rGO scaffolds. The scanning temperature ranged from 25 to 250°C at a rate of 10°C/min in a hermetically sealed in aluminum plate (flow rate of 20 ml/min). Crystallinity (*Xc*) was calculated by the (formula 3) [7].

$$Xc = \frac{(\Delta Hm - \Delta Hc)}{\Delta Hmp} \times 100\% \quad (3)$$

where *ΔHm* is the heat of fusion (J/g); *ΔHc* is the heat of cold crystallization (J/g); *ΔHmp* is the heat of fusion of 100% crystalline PLLA (93,6 J/g).

2.2.8 XRD

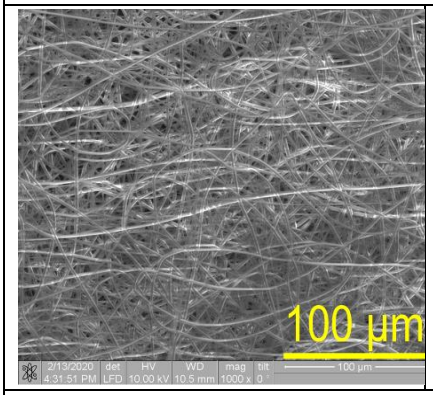
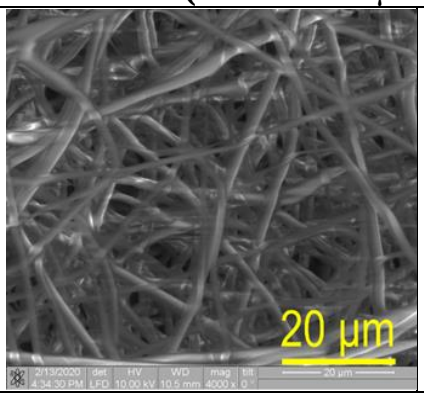
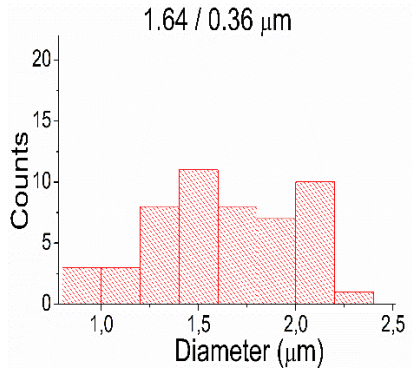
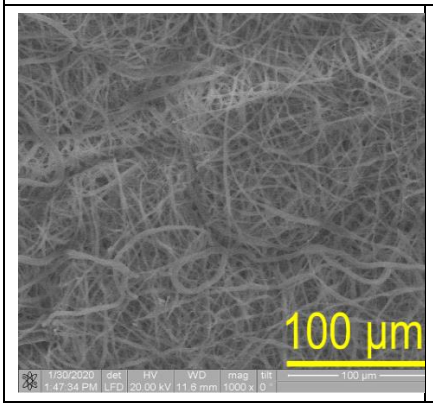
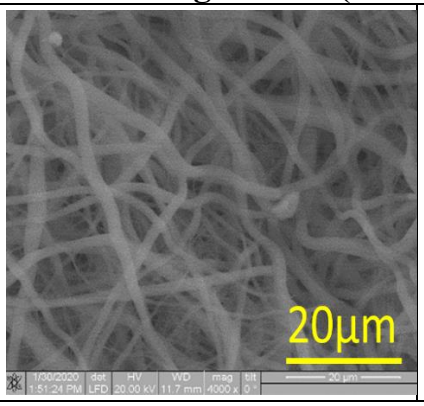
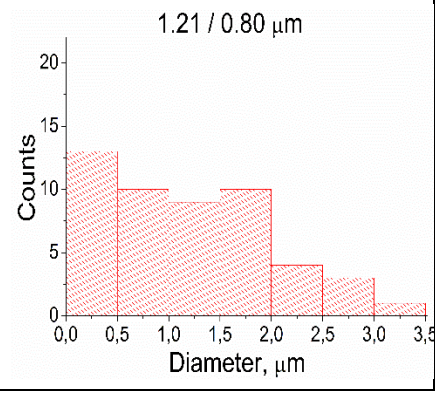
The effect of GO and rGO additives on the phase composition and crystal structure of PLLA scaffolds was studied using a XRD–6000 diffractometer (Shimadzu Corporation, Japan) in the automatic mode in the range of scattering angles from 5 to 80°.

Chapter 3. Results and Discussions

3.1 Morphology analysis of the PLLA scaffolds before and after annealing

In order to alter the material properties, annealing can be defined as a process where a material undergoes a heat treatment at a certain temperature, which is kept there for a definite time. Afterwards, a material (sample) is cooled down until the room temperature. The annealing of semicrystalline polymers may change the crystal structure, the degree of crystallinity, the orientation of both crystalline and amorphous phases, their contiguous structural morphology, and the number of tie chains between the crystallites [79]. For amorphous polymers, the thermal treatment can play a very important role in changing the morphology of the polymer [79].

Table 3– SEM images of PLLA scaffolds before and after annealing

Image (1000X)	Image (4000X)	Fiber size (µm)
Pure PLLA (1.64 ± 0.36 µm)		
		
Pure PLLA Annealing 160 °C (1.21 ± 0.8 µm)		
		

To investigate the effect of annealing on the PLLA scaffolds morphology, SEM analysis was done (Table 3). As it can be seen, the heat treatment caused a significant shrinkage of the fibers, and largely curled fibers were observed after annealing. This

phenomenon is explained due to the relaxation of oriented amorphous PLLA chains at temperatures above glass temperature [80].

3.2 Investigation of the rGO/GO content influence on the morphology of the PLLA scaffolds

SEM was also applied to examine the morphology of the prepared PLLA–rGO and PLLA–GO composite scaffolds (tables 4 and 5). It is clearly shown that PLLA–GO/rGO composite fibers were successfully prepared through electrospinning. The formed fibers indicated that a relatively fine dispersion of rGO/GO fillers (no defects) in the PLLA polymer matrix was achieved. The fiber morphology shows tendency toward a decrease in the average fiber diameter with an increase in the rGO/GO content in the polymer matrix from 1.64 ± 0.38 μm for pure PLLA to 1.02 ± 0.40 μm for PLLA 1wt % rGO (by 38 %), and up to 1.12 ± 0.25 μm for PLLA 1 wt% GO (by 32 %). This may be due to a change in the physicochemical properties of the electrospinning solution under the influence of rGO/GO additives, such as the large charge accumulations in the solution jets caused by the abundant charges on the surfaces, leading to strong electrostatic repulsions [81–84]. In addition, it is observed that PLLA–rGO contributes more porosity structure than PLLA–GO.

Table 4 – SEM images of PLLA scaffolds with different contents rGO

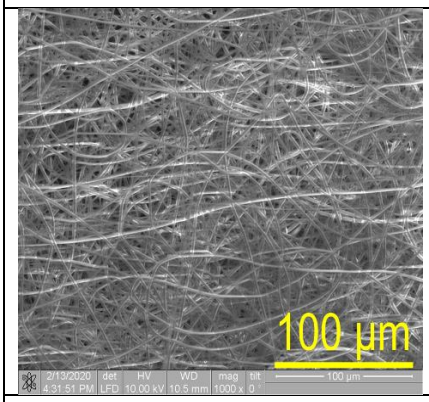
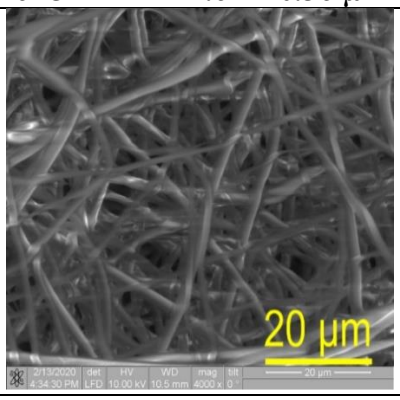
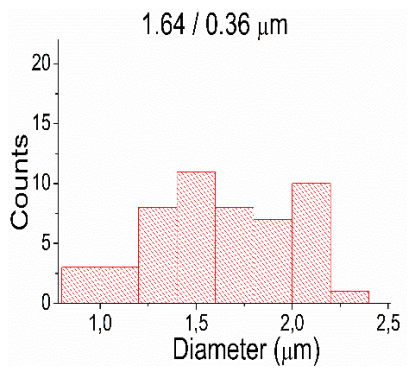
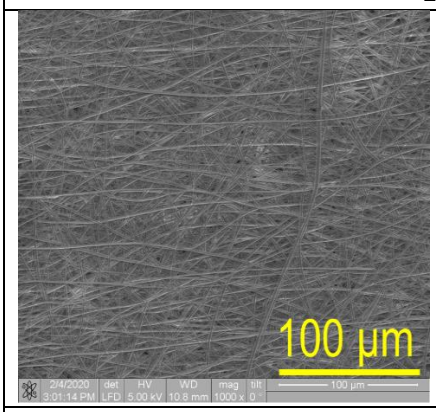
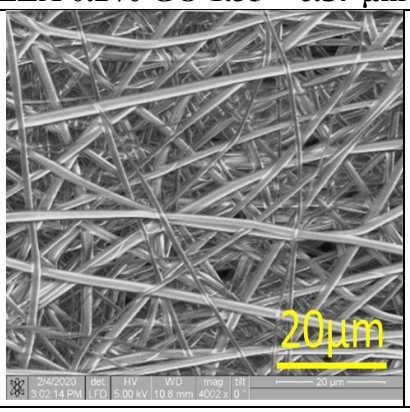
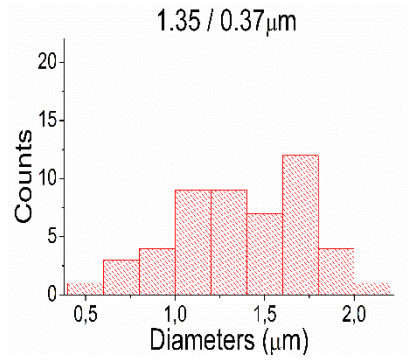
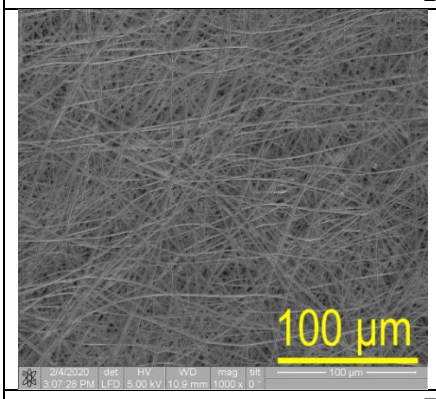
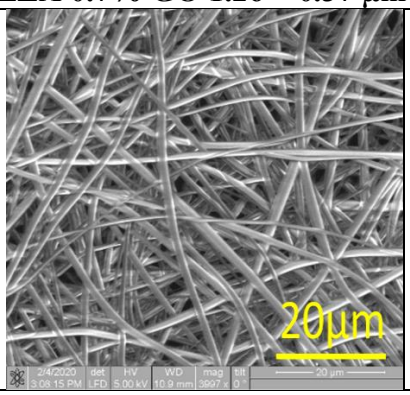
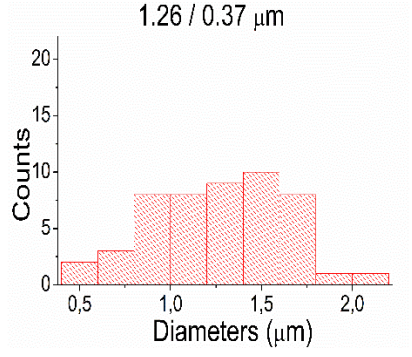
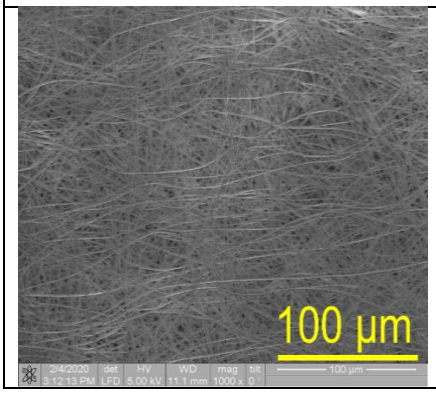
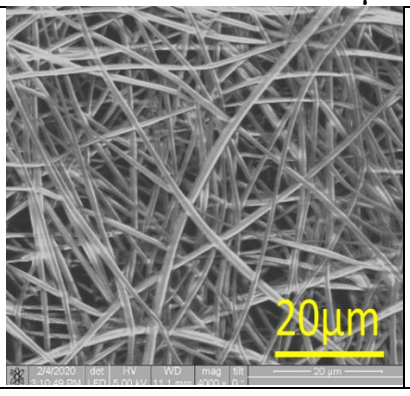
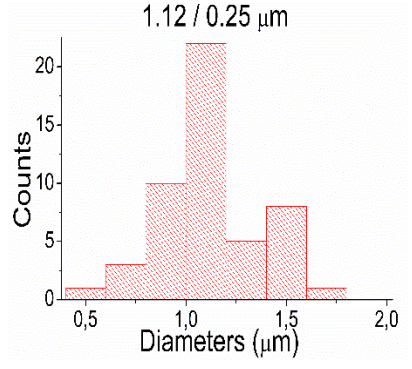
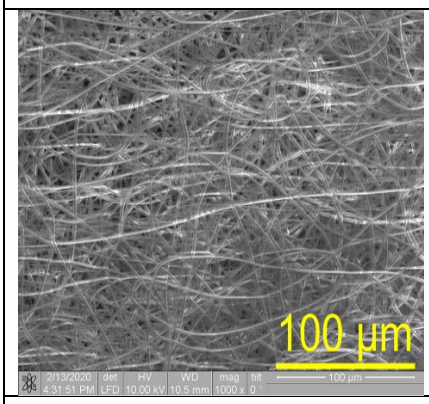
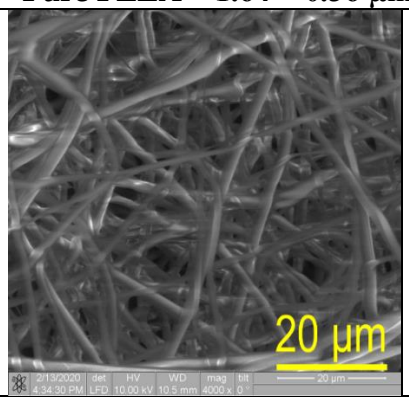
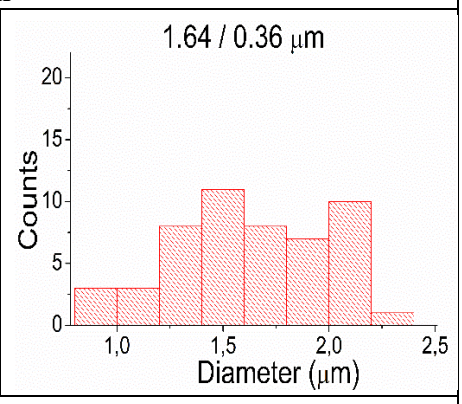
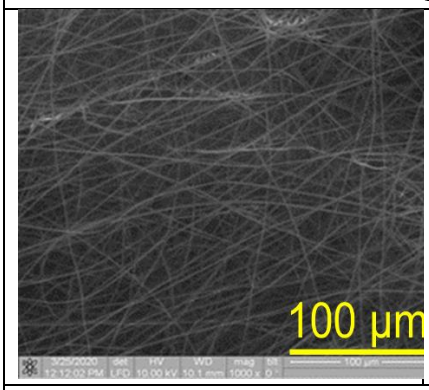
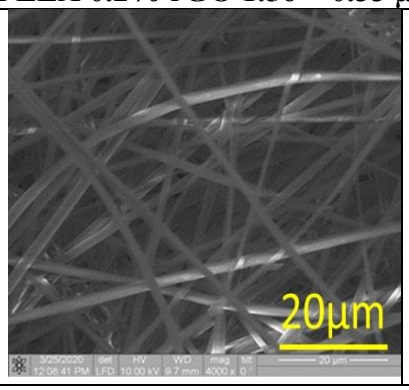
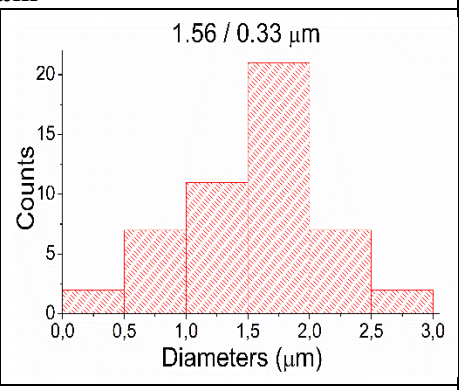
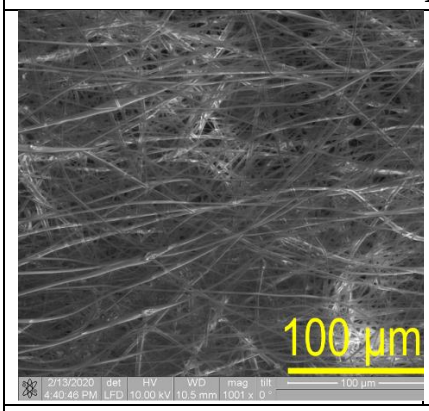
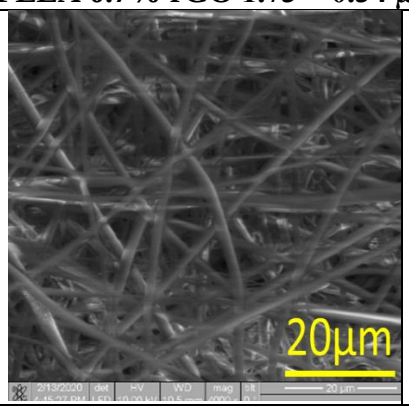
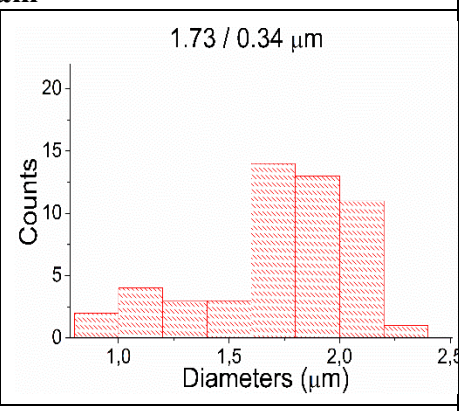
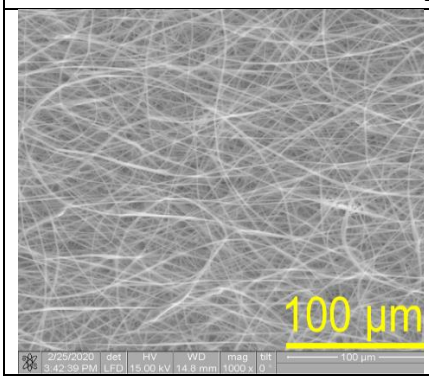
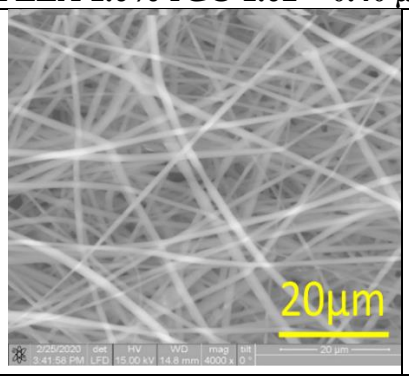
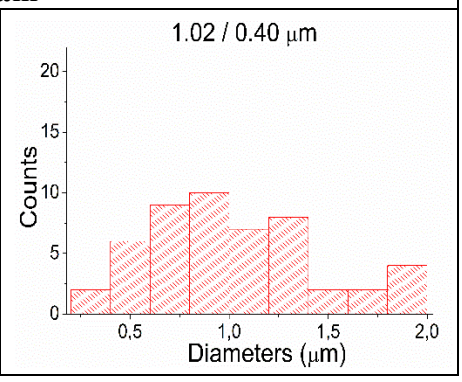
Image (1000X)	Image (4000X)	Fiber size(µm)
Pure PLLA 1.64 ± 0.36 µm		
		
PLLA 0.2% GO 1.35 ± 0.37 µm		
		
PLLA 0.7% GO 1.26 ± 0.37 µm		
		
PLLA 1.0% GO 1.12 ± 0.25 µm		
		

Table 5 – SEM images of PLLA scaffolds with different contents GO

Image (1000X)	Image (4000X)	Fiber size(μm)
Pure PLLA 1.64 ± 0.36 μm		
		
PLLA 0.2% rGO 1.56 ± 0.33 μm		
		
PLLA 0.7% rGO 1.73 ± 0.34 μm		
		
PLLA 1.0% rGO 1.02 ± 0.40 μm		
		

3.3 Investigation of annealing impact on the molecular structure of PLLA scaffolds

The molecular bonds of the PLLA scaffold samples were studied using FTIR. Figure 6 shows the IR spectra of PLLA fibers before and after annealing at 160°C in the range from 900 to 1800 cm^{-1}. The measurements were carried out at several points located in different places of scaffolds to show the identity of the composition along the entire surface. Infrared analysis revealed the presence of standard PLLA absorption bands listed in Table A1 (appendix A) [17, 19]. The characteristic deformation vibrations of the groups of symmetric and asymmetric functional groups CH_3 were detected in the range C=O– (2920–2850 cm^{-1}) and C–O– (1735–1758 cm^{-1}), respectively. Symmetric and asymmetric CH_3 vibrations were also observed in the range of about 1357–1450 cm^{-1}, respectively.

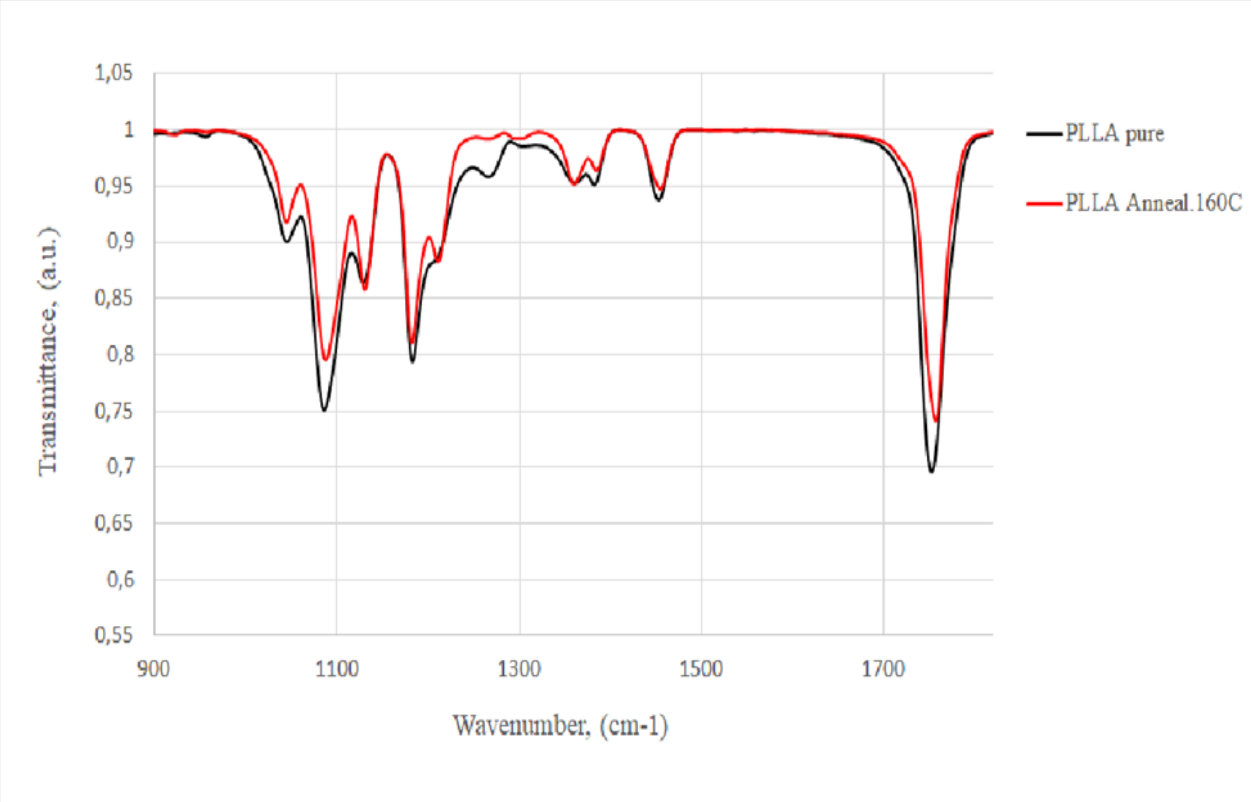


Figure 6 – FTIR of PLLA scaffolds before and after annealing

In IR fingerprint range from 900 to 1000 cm^{-1} (figure 7), a decrease in the intensity of the band at 955 cm^{-1} corresponding to amorphous phase of PLLA and an increase in intensity at 921 cm^{-1} in corresponding to α-phase of PLLA after annealing was observed [19], thus, resulted in the changed phase composition of scaffolds.

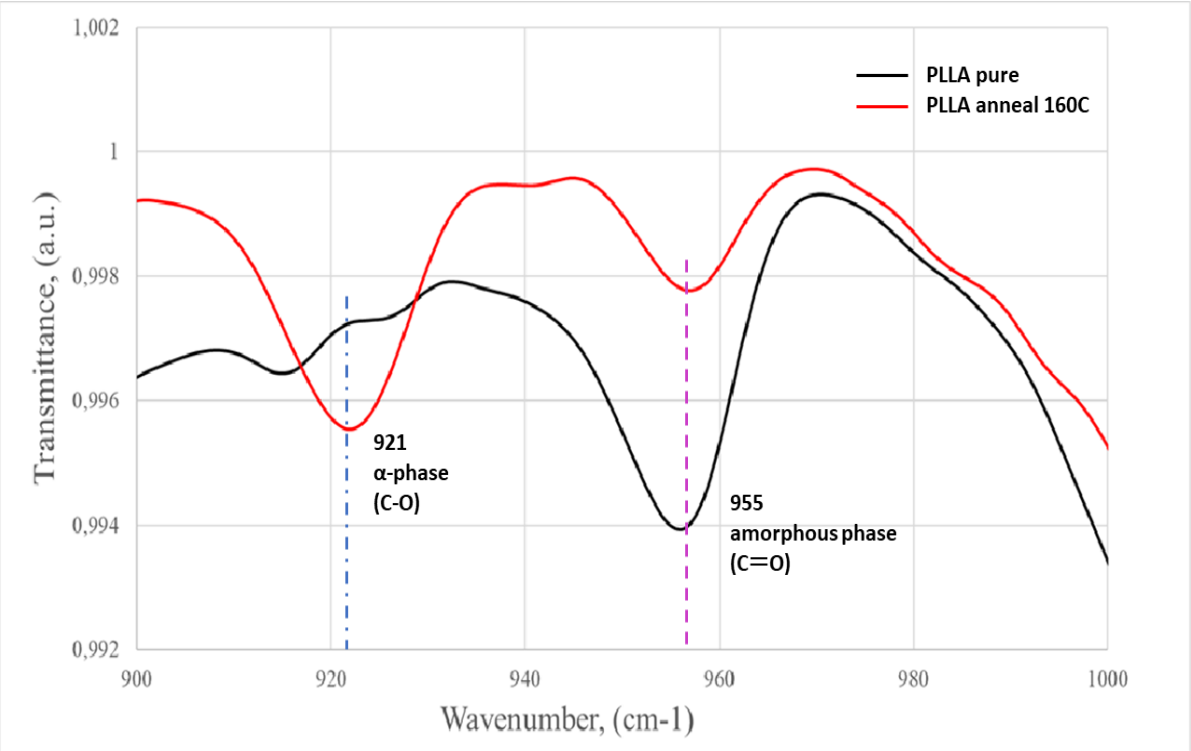


Figure 7 – FTIR of PLLA scaffolds before and after annealing

3.4 FTIR analysis of PLLA rGO/GO scaffolds

FTIR was used to study the state of molecular bonds depending on the structure of the obtained rGO/GO composites. Figure 8 shows the FTIR spectrum of PLLA–GO/rGO from the (range 1800–525cm^{-1}).

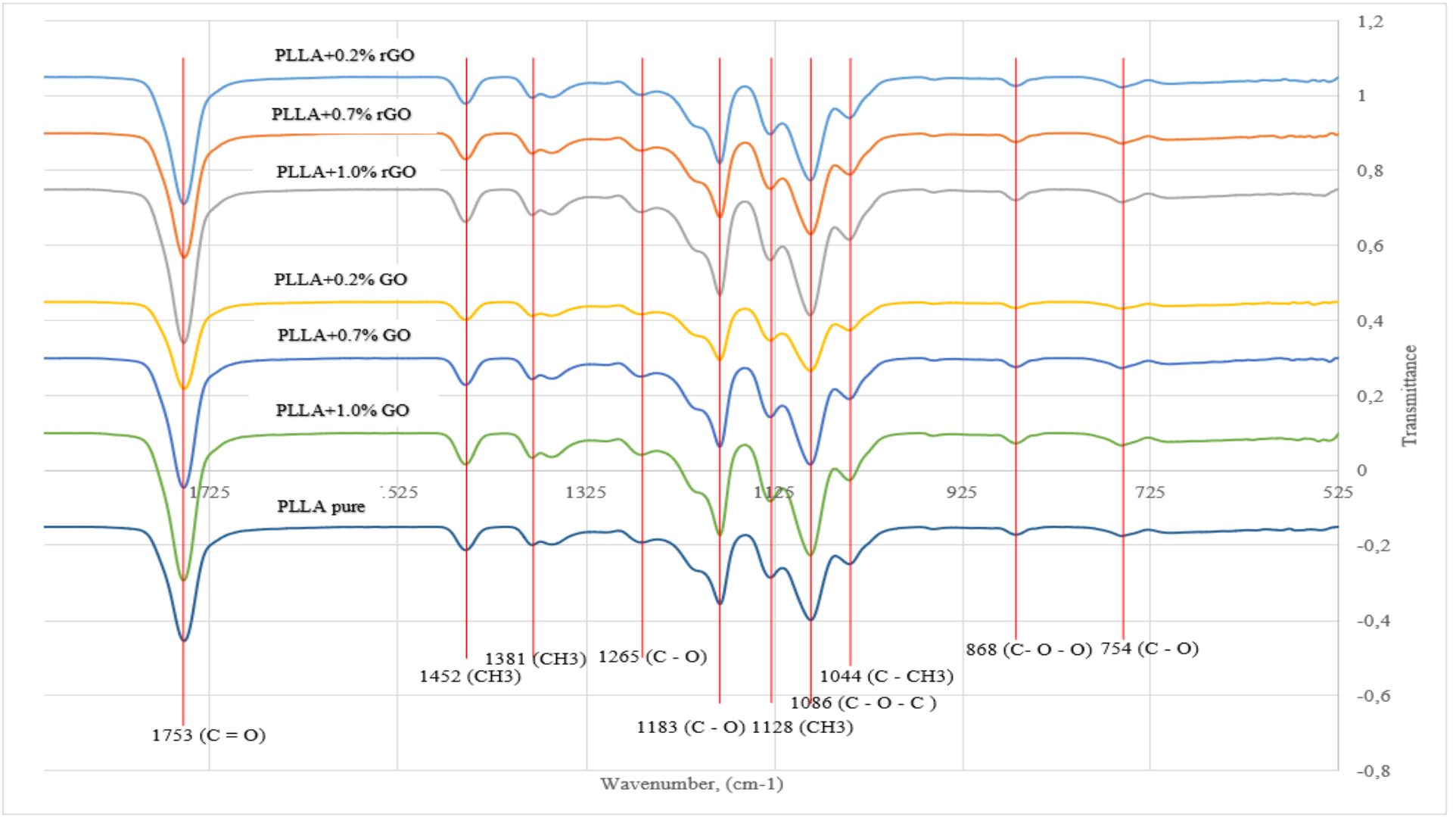


Figure 8 – FTIR of PLLA scaffolds with different contents of GO/rGO (range of 1800–525cm-1)

To estimate the impact of rGO/GO nanofillers on the PLLA phase composition, the FTIR spectra were carefully analyzed in the fingerprint range of 900–1000 cm^{-1}. As it can be seen (Figure 9), pure and hybrid PLLA scaffolds with 0.2 wt.% and 1.0 wt.% rGO had a similar spectral profile, i.e. showing a low intensive peak at about 915–916 cm^{-1} corresponding to the α' meso-crystalline phase [85]. In turn, 0.7 wt.% rGO demonstrated two low intensive peaks at about 921 cm^{-1} and 912 cm^{-1} which corresponds to the α- and β-phases of PLLA, respectively [84].

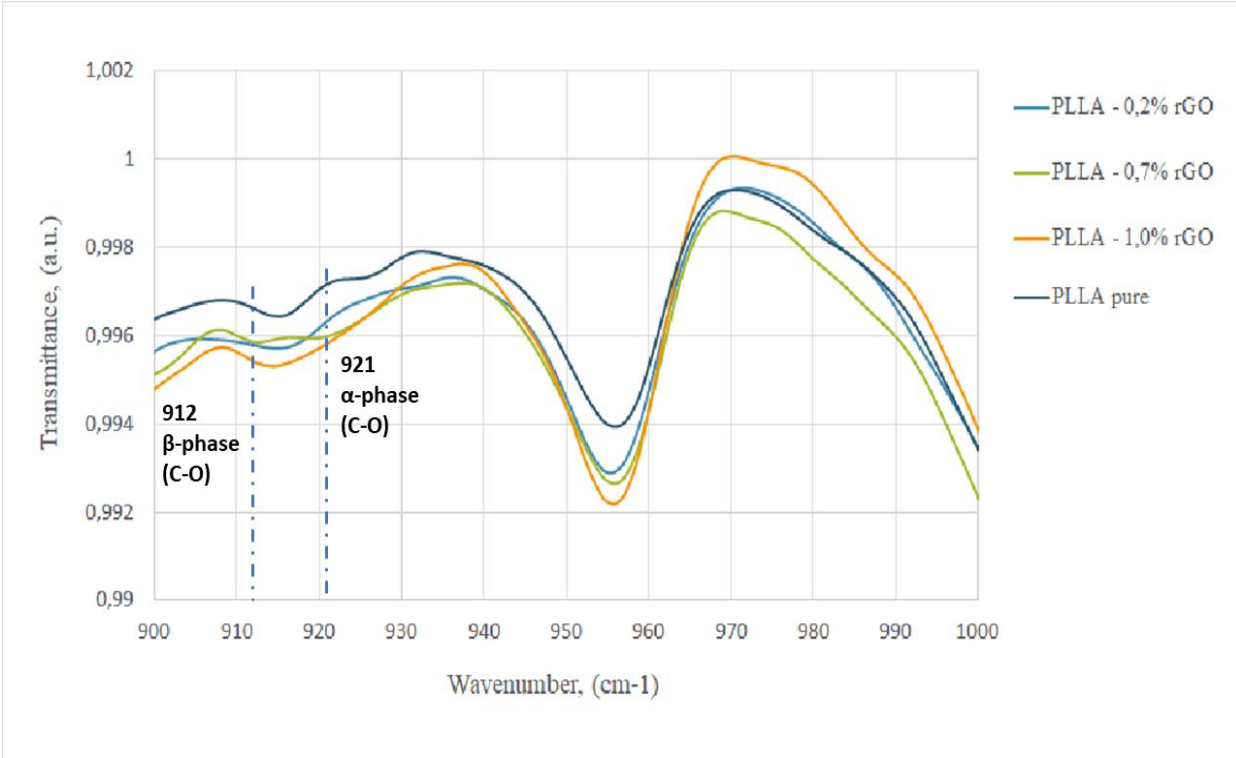


Figure 9 – FTIR of PLLA scaffolds with different contents of rGO (range of 900-1000 cm-1)

Meanwhile, hybrid PLLA–GO scaffolds showed similar changes in FTIR spectra as in the case of rGO doping (Figure 10). Pure and hybrid scaffolds with doping 0.2 wt.% and 1.0 wt.% GO also demonstrated a low intensive peak at about 915–916 cm^{-1} corresponding to the α' meso–crystalline phase. The addition of 0.7 wt.% GO resulted in two peaks at about 921 cm^{-1} and 912 cm^{-1}, which was attributed to the α- and β-phases of PLLA [86].

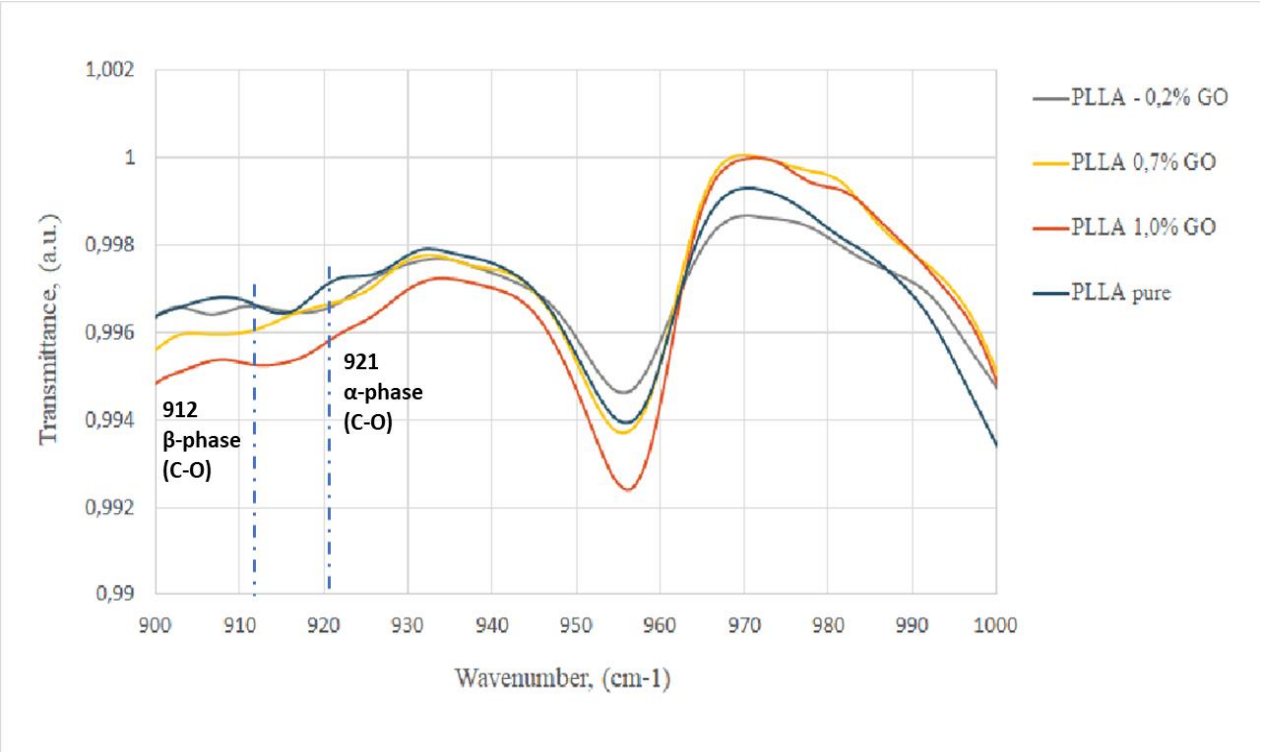


Figure 10 – FTIR of PLLA scaffolds with different contents of GO (range of 900–1000 cm−1)

In short, the addition of 0.7 wt.% GO/rGO in the PLLA fibers demonstrated the presence of β- (912 cm^{-1}) and α- phases (921 cm^{-1}) in PLLA polymer matrix, thereby the presence of the piezoelectric response can be expected for hybrid scaffolds.

3.5 TGA and DSC analysis of PLLA–GO/rGO

Thermal stability is an important property for polymer composites. The TGA curves for pure PLLA and PLLA–GO/rGO composites are illustrated in figure 11, 12. It can be seen that the decomposition temperature of the composites commences at around 250°C and rapidly continues until 400°C. A slight shift in thermal stability for PLLA 0.2 wt.% rGO scaffolds can be observed and its decomposition temperature starts at 210°C and ends at 420°C. Notice that from the graph the addition of fillers (GO/rGO) does not affect the thermal stability of the PLLA polymer matrix. This probably due to the low rGO/GO content in the PLLA matrix [87–89].

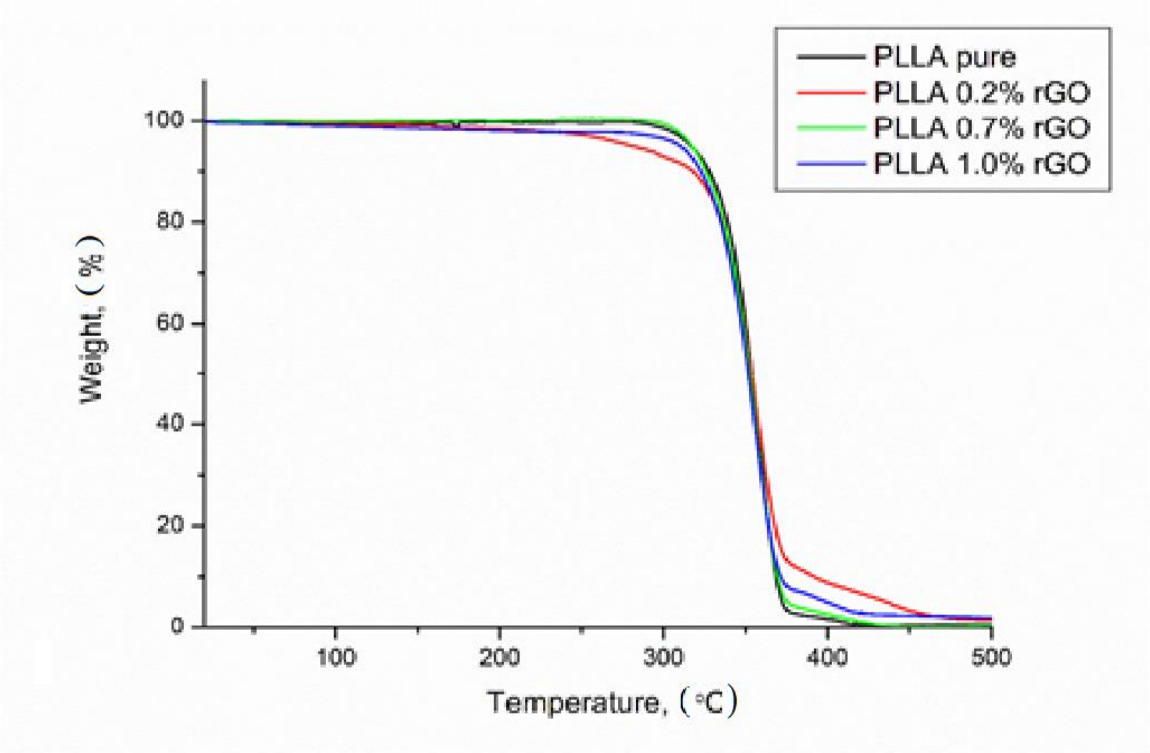


Figure 11 – TGA curves for PLLA scaffolds with different contents of rGO

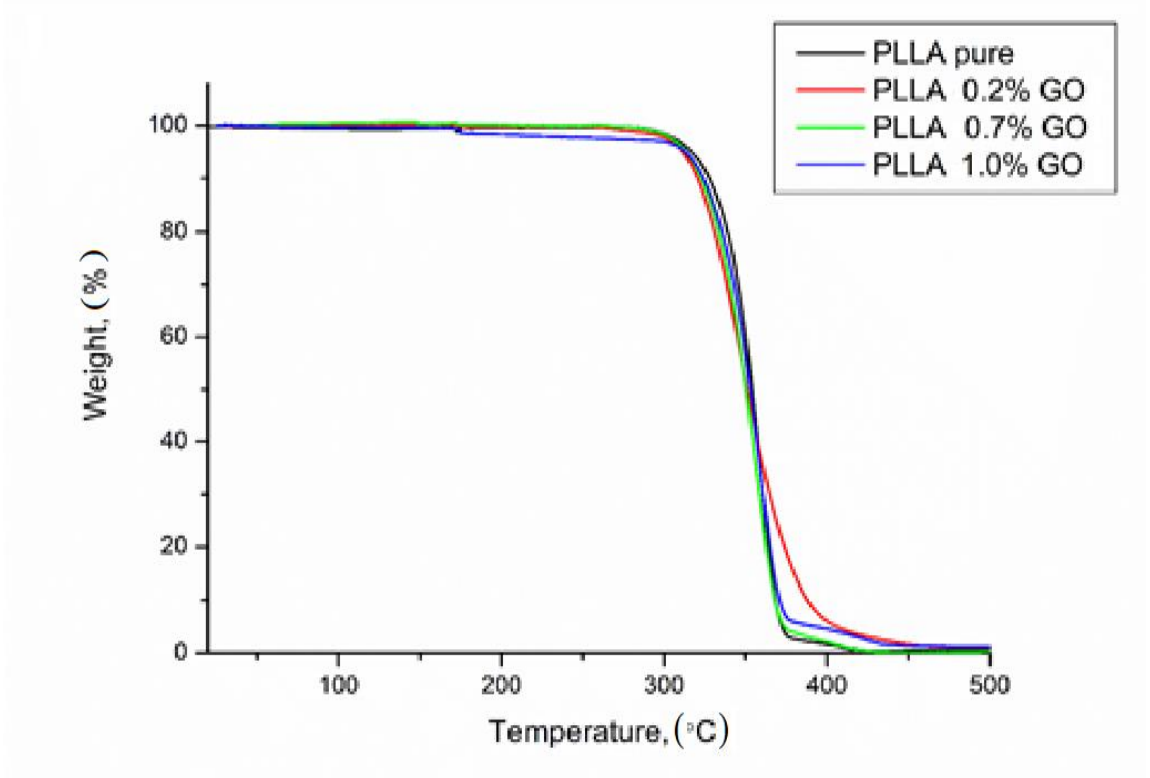


Figure 12– TGA curves for PLLA scaffolds with different contents of GO

DSC was used to investigate the thermal behavior of the pure PLLA and PLLA –GO/rGO composites scaffolds. The resulting temperature curves are presented in the figures 13 and 14.

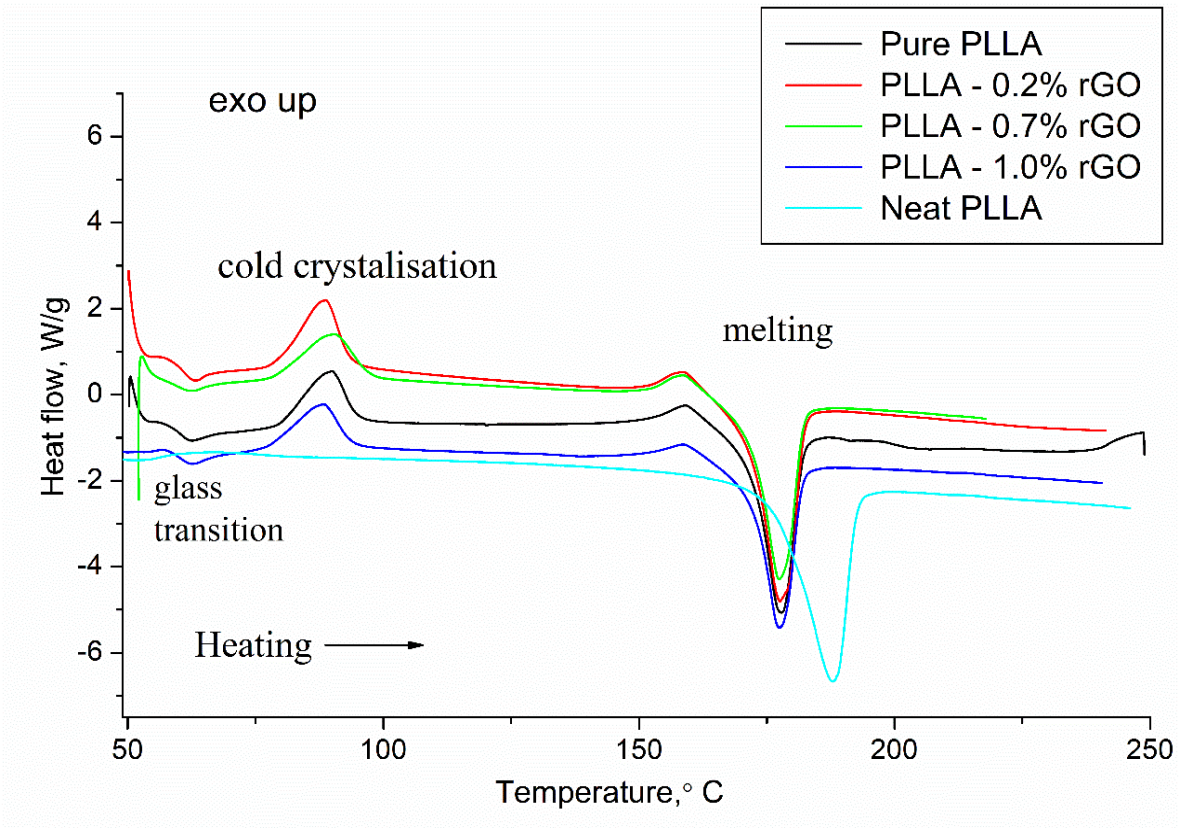


Figure 13 – DSC heating curves for neat PLLA and PLLA–rGO composite scaffolds

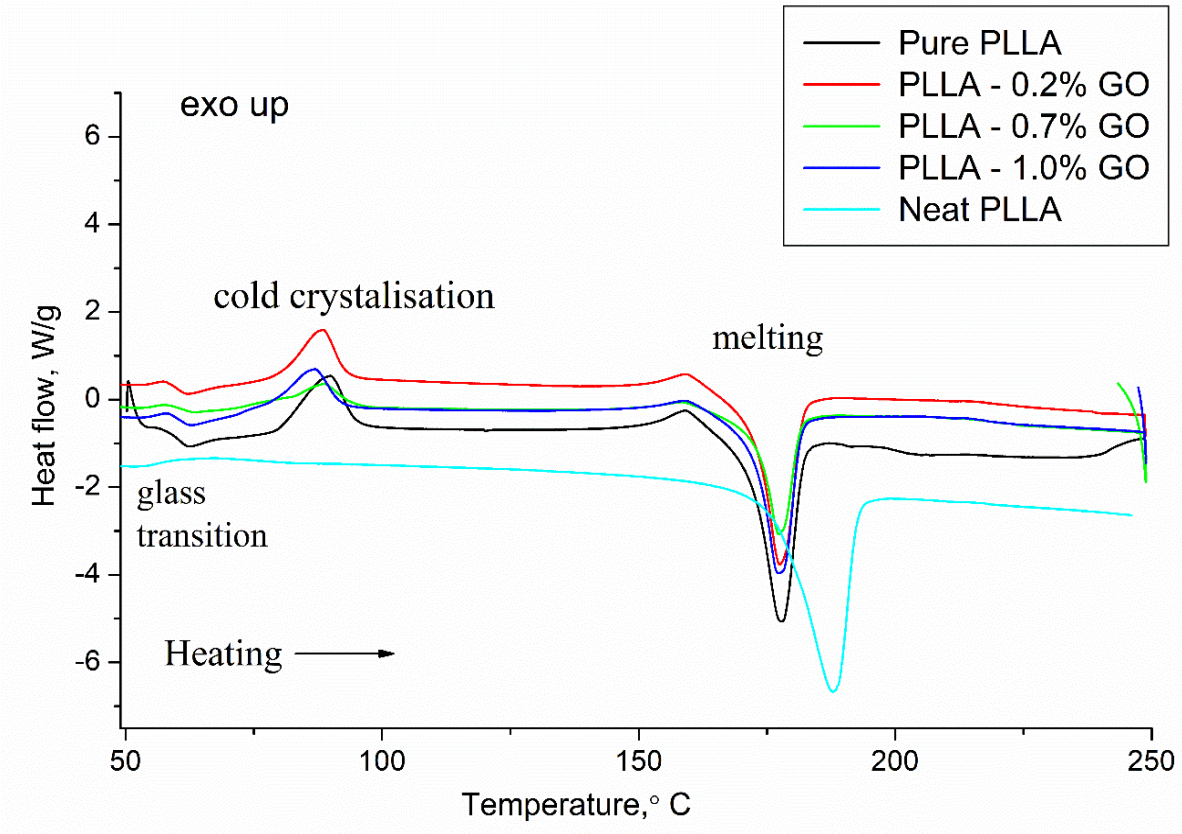


Figure 14 – DSC heating curves for neat PLLA and PLLA–GO composite scaffolds

Thermal characteristics and crystallinity degree of pure PLLA and PLLA–GO/rGO nanocomposites are summarized in table 6.

Table 6 – DSC parameters for heating process Pure PLLA, PLLA–rGO/GO

PLLA sample	Tg, °C	ΔHg, J/gr	Tcc1, °C	ΔHcc1, J/g	Tm, °C	ΔHm, J/g	***Xc,* %**	Average fiber diameter, (μm)
Pure	61.9	4.18	89.7	24.77	177.7	69.84	**48.15**	1.64 ± 0.36
0.2 %rGO	62.7	3.33	88.7	22.27	177.5	64.19	**44.79**	1.56 ± 0.33
0.7 %rGO	61.8	4.18	90.5	21.90	177.3	65.00	**46.05**	1.73 ± 0.34
1.0 %rGO	62.5	4.32	88.3	20.91	177.4	61.11	**42.95**	1.02 ± 0.40
0.2 % GO	62.0	3.61	88.5	19.95	177.5	62.40	**45.35**	1.35 ± 0.37
0.7 % GO	63.8	4.41	88.8	13.14	177.2	54.90	**44.62**	1.26 ± 0.37
1.0 % GO	62.8	3.93	86.9	19.55	177.1	59.66	**42.74**	1.12 ± 0.25
Neat	52.9–66.5	-	-	-	187.8	87.89	**-**	-

Estimating the obtained DSC results for PLLA scaffolds containing 0.2 wt %, 0.7 wt %, 1.0 wt % rGO/GO. It is noted that with an increase in the concentration of additives, insignificant changes in the temperature characteristics occurs in the samples. A slight increase in the glass transition temperature is observed for composite samples Tg up to 62.7°C for PLLA–rGO and up to 63.8°C for PLLA–GO, which may be due to some limiting effect of rGO/GO on the molecular mobility of PLLA. Glass transition temperature (Tg) is commonly identified as a complex phenomenon that relies on several factors such as intermolecular interaction, chain flexibility and molecular weight of the material itself [90–93]. It is noted, by an increase in rGO/GO content, nanocomposites show a slight decrease in melting temperature (Tm) from 177.7°C to 177.4°C for PLLA 1 wt.% rGO and up to 177.1°C for PLLA 1 wt.% GO. Moreover, the melting peaks of composite scaffolds, upon closer examination (figure.15, 16), have a slightly different peaks from pure PLLA (the melting peaks looks as a parabola curve).

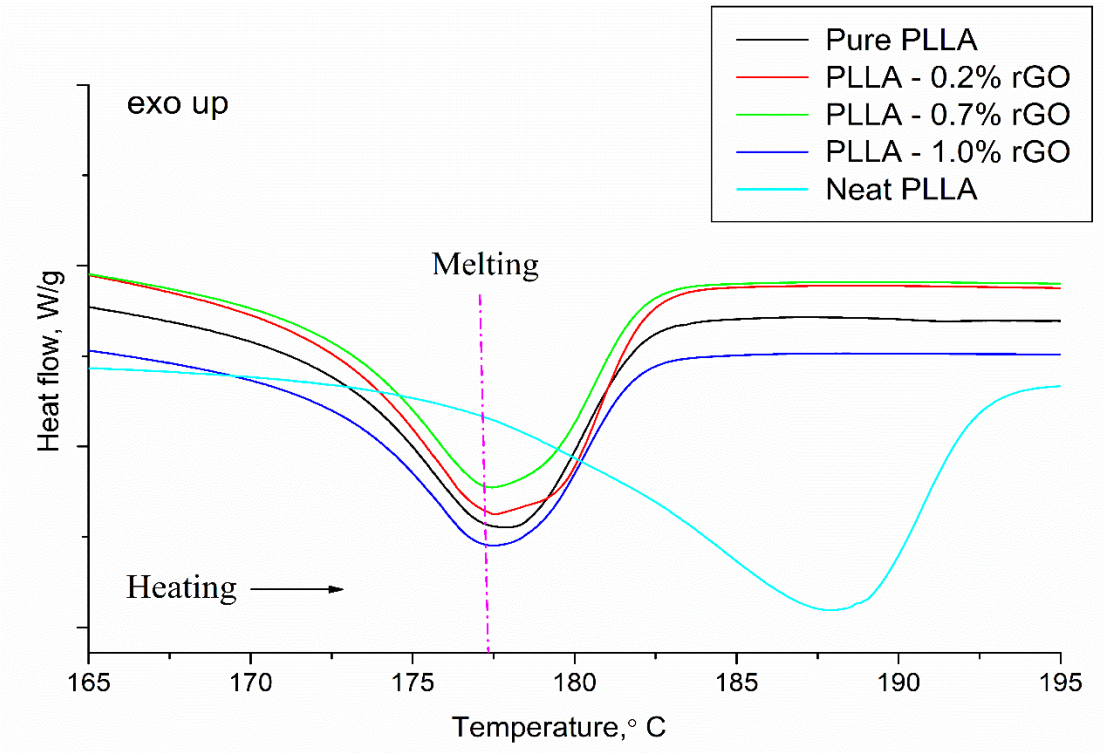


Figure 15 – DSC melting curves for neat PLLA and PLLA–rGO composite scaffolds

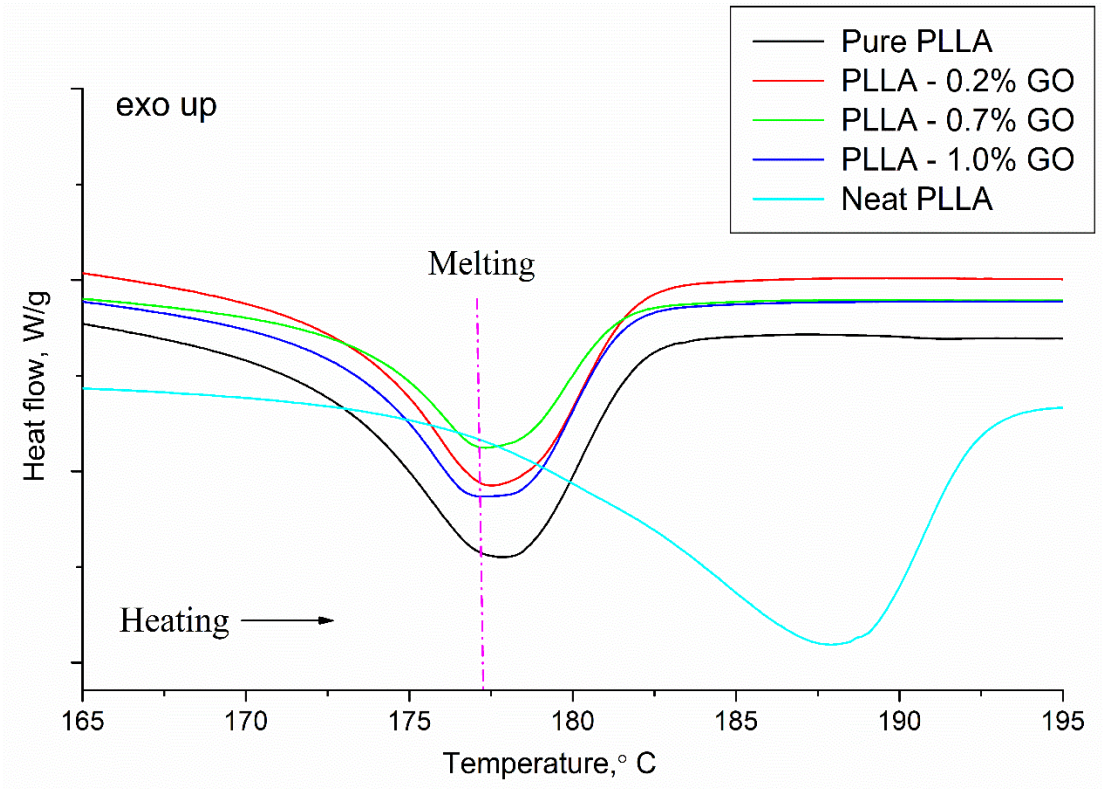


Figure 16 – DSC melting curves for neat PLLA and PLLA–rGO composite scaffolds

The peaks of 0.7 wt % and 1.0 wt% rGO composites are shifted toward lower temperatures. These differences reveal the changes that occur during the formation of

rGO/GO composites in a supramolecular structure that determines the physical characteristics of the polymer [94]. The crystallinity values (*Xc*), 48.15% for pure PLLA, decreases to 42.95% for PLLA 1 wt % rGO and to 42.74% for PLLA 1 wt % GO (tables 7). There are a clear correlation and a direct relationship between *Xc* and the average fiber diameter of the composites, which in turn depends on the parameters of the electrospinning process, which change as a result of an increase in the content of rGO/GO additives. Thus, as the rGO/GO content of the composite increases, the average fiber diameter decreases and the degree of crystallinity also decreases [93–96]. All the scaffolds showed obvious exothermic peaks Tcc1 and Tcc2 which is evidence of the presence of a crystalline phase in the samples. In the amorphous Neat PLLA Tcc1peaks are not visible, it has only Tcc2 peak (figure 17, 18). For pure PLLA scaffold, Tcc1 = 89.7°C and further, with a slight decrease in temperature with an increase in rGO/GO content to Tcc1 = 88.3°C for PLLA/rGO and to Tcc1 = 86.9°C for PLLA/GO (table 7 and figure 17, 18).

Table 7 – DSC parameters for cooling process pure PLLA, PLLA–GO/GO

PLLA samples Wt (%)	Tcc1, °C	ΔHcc1, J/g	Tcc_2, °C	ΔHcc2, J/g	Xc, %
Pure	89.7	24.77	113.6	42.02	**48.15**
0.2 % rGO	88.7	22.27	115.5	44.27	**44.79**
0.7 % rGO	90.5	21.90	113.6	39.56	**46.05**
1.0 % rGO	88.3	20.91	117.2	39.11	**42.95**
0.2 % GO	88.5	19.95	112.9	39.11	**45.35**
0.7 % GO	88.8	13.14	115.2	32.53	**44.62**
1.0 % GO	86.9	19.55	114.2	37.17	**42.74**
Neat	-	-	109.7	15.23	-

A decrease in Tcc1 indicates the occurrence of PLLA chain mobility at lower temperature due to an increase in the contact surface with the heating medium, since the diameter of the resulting fibers decreases with increasing additive content. But it is also possible that this is due to the presence of rGO/GO additives leads to a greater decrease in temperature (by 2.8°C), which is consistent with the results of [97–99].

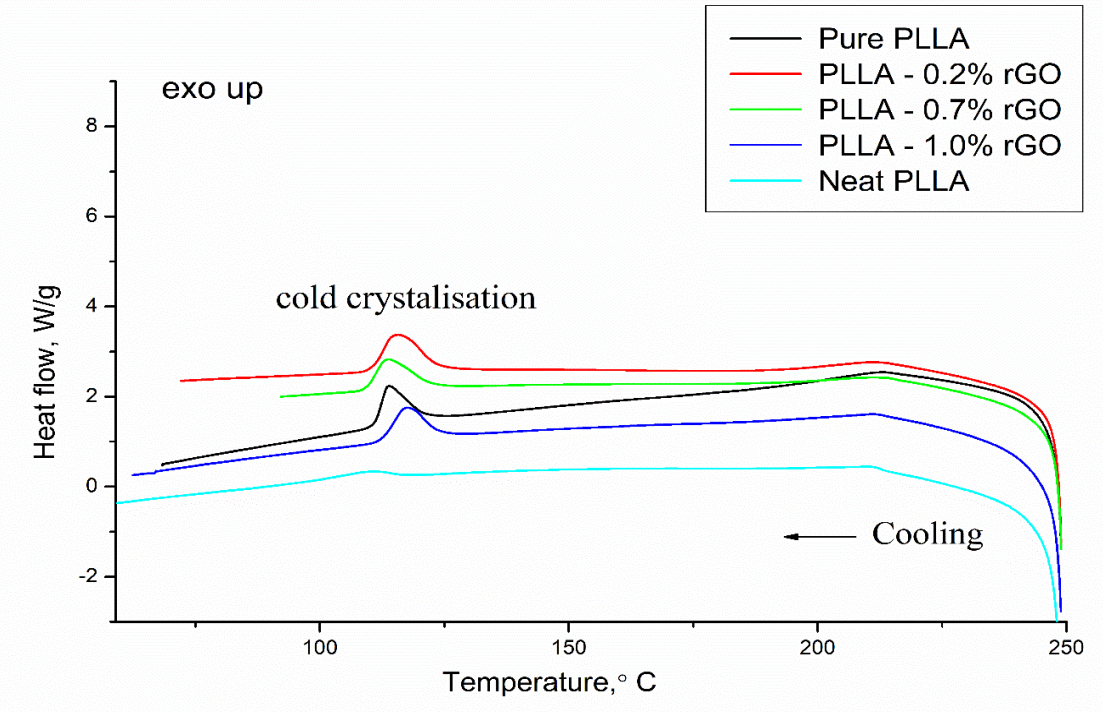


Figure 17 – DSC cooling curves for neat PLLA and PLLA–rGO scaffolds

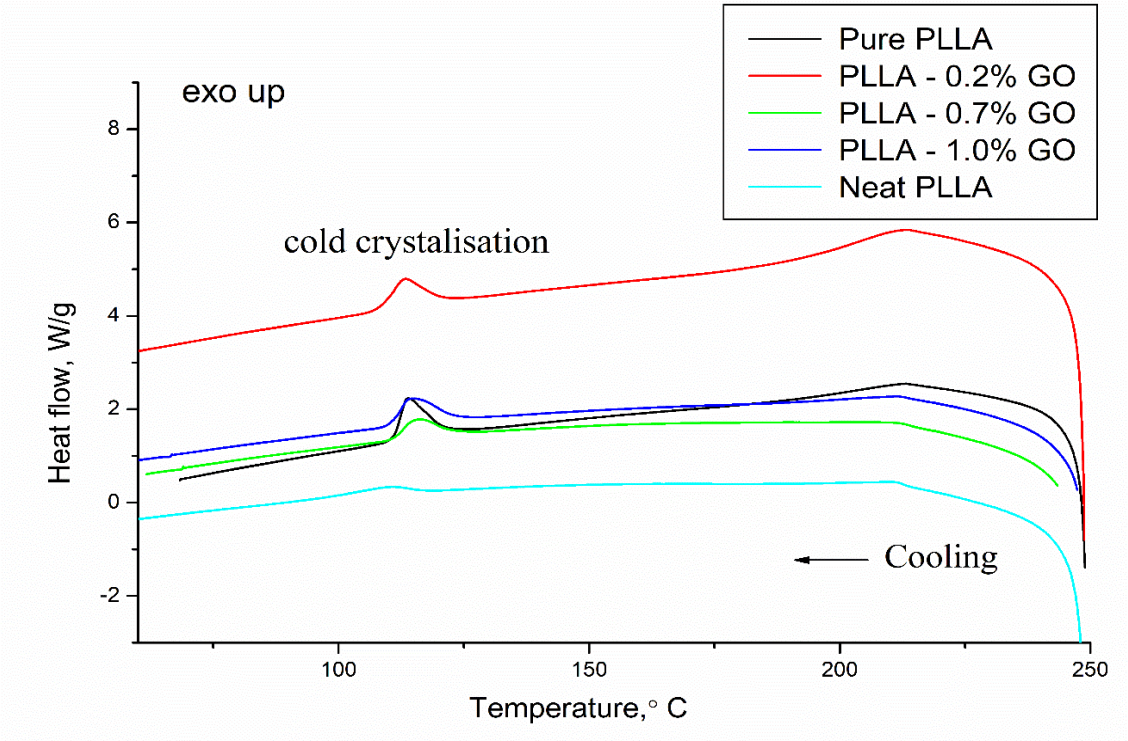


Figure 18 – DSC cooling curves for neat PLLA and PLLA–GO scaffolds

It is also noted that rGO/GO have an effect on Tcc2 during the melt cooling process. For pure PLLA, Tcc2 = 113.6°C and with an increase in the content of

additives Tcc2 varies from 115.5 to 117.2°C for PLLA/rGO and 112.2 to 115.2°C for PLLA/GO (table 7). These temperature changes indicate that the presence of rGO/GO promotes non–isothermal crystallization of PLLA matrix [100–102]. The addition of rGO/GO fillers affects the kinetics of the formation of the PLLA crystal lattice at certain concentrations promoting the formation of various crystalline configurations, but the addition of rGO/GO also affects the parameters of the electrospinning process by changing the viscosity and conductivity of the solutions, affecting the diameter of the fibers, as evidenced by the SEM results. A decrease in the diameter of the fibers negatively affects the formation of the total amount of the crystalline phase in the composites [103]. All the obtained PLLA rGO/GO composites have a semi–crystalline structure and their Xc is about 43–46%. The highest Xc = 48.15% for pure PLLA scaffold fibers.

3.6 XRD analysis of PLLA before and after annealing

X-ray diffraction measurement is one of the few characterization techniques that are able to differentiate the α- and β- crystalline phases. Figure 19 shows the XRD pattern of Pure PLLA, it clearly demonstrates the presences of dominant amorphous phase and nanocrystalline structure [24, 7]. In the process of electrospinning, under the influence of a strong electric field, PLLA molecules are polarized and a large number of nanocrystallites are formed, which is observed in the presented diffraction patterns. It is also possible that the high molecular weight of PLLA in the studied samples (about Mw 180,000 g/mol) limits the mobility of the molecular chains and thereby complicates the formation of crystalline structures (crystallites) directly during electrospinning [102]. Hence, to increase its crystallinity structure various techniques such as annealing and various nucleating agents are approached. Temperature is a critical parameter that affects the crystallization properties of the polymer matrix. An increase in temperature leads to an increase in the chain mobility creates the possibility for the reorganization of the crystalline structure of the polymer [82, 83]. An increase in the degree of crystallinity of pure PLLA after annealing (160°C) is observed in the obtained XRD pattern (figure 16).

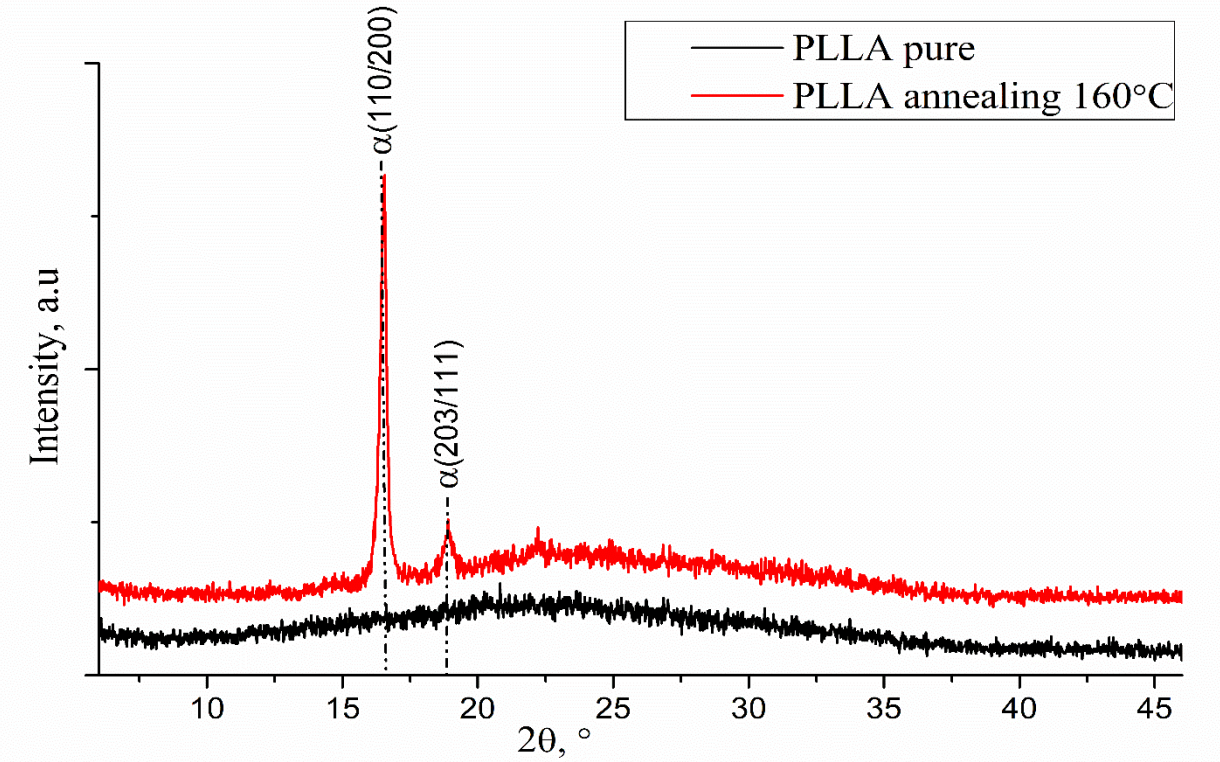


Figure 19 – XRD patterns of pure PLLA and PLLA annealing (160°C) scaffolds

X–ray diffraction analysis revealed the presence of a high narrow peak at 2θ ≈ 16.6° and a small peak at 2θ ≈ 19.0°, which corresponds to the PLLA α- phase (card № 00–054–1917, figure. B1, appendix B). Since the PLLA α- phase does not show significant piezoelectric compression response [56, 103] hence this method has any respective of our goal. Further, the nucleating agents were used.

3.7 XRD analysis of PLLA-rGO/GO scaffolds

To study the crystallographic structure of the GO/rGO fillers on PLLA matrix, XRD analysis was performed. Figure 20, 21 represents typical XRD patterns of the PLLA scaffolds before and after GO/rGO fillers.

Based on the XRD patterns pure PLLA and PLLA–rGO/GO 0.2 wt % showed the presence of a halo and the absence of characteristic reflections in the diffraction patterns, which probably indicates the presence of a predominantly nanocrystalline structure in the samples obtained.

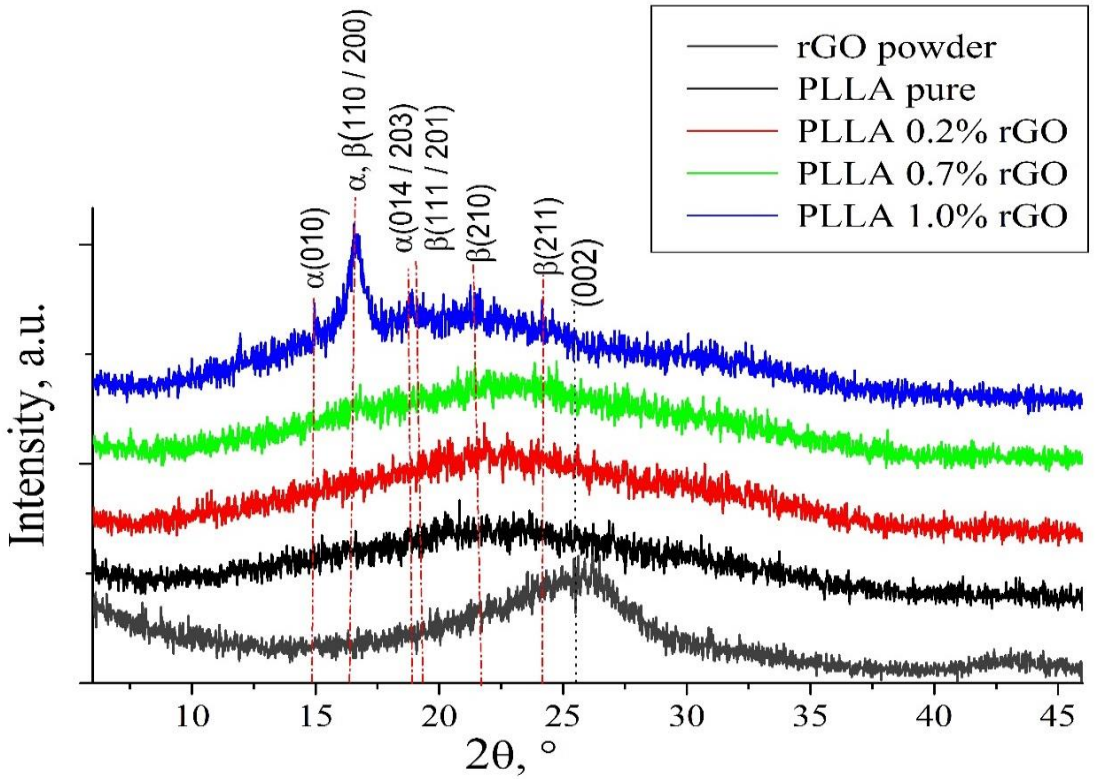


Figure 20 – XRD patterns of pure PLLA, rGO powder and PLLA–rGO composite

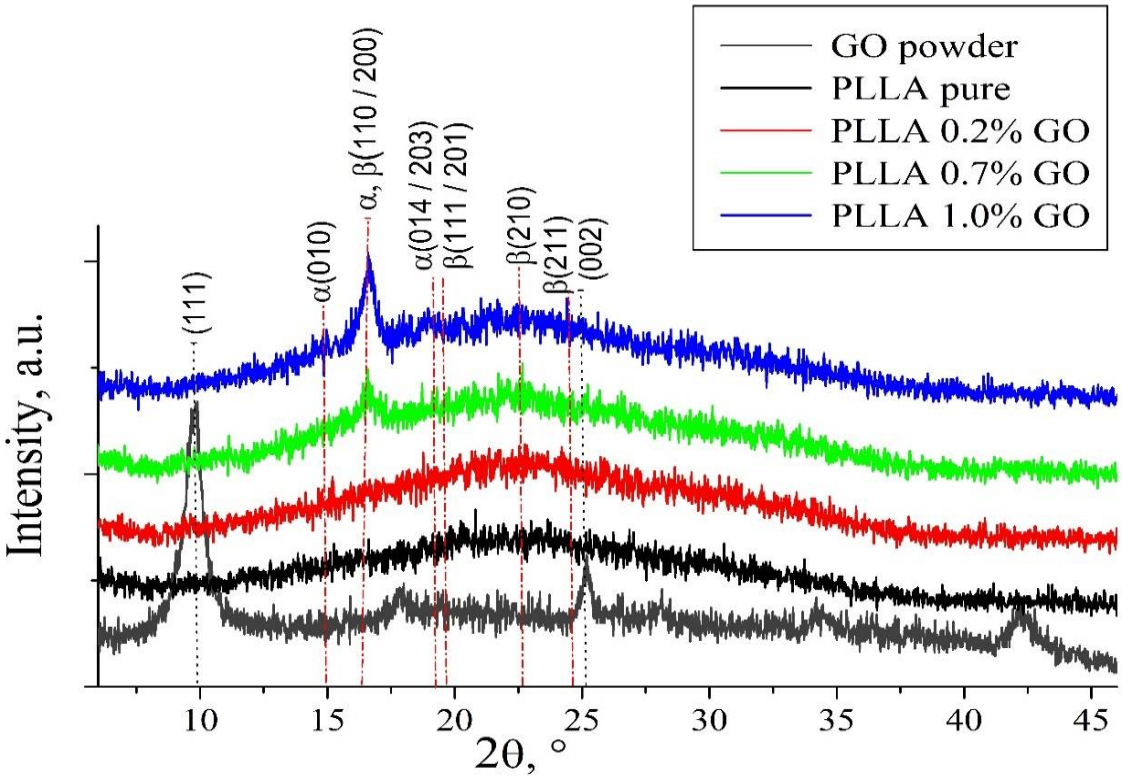


Figure 21 – XRD patterns of pure PLLA, GO powder, and PLLA–GO composites

The 0.7 wt % and 1 wt % rGO/GO fillers in the PLLA matrix led to the appearance of important characteristic reflections of the crystalline phase of PLLA, observed at 2θ ≈ 15.0° (010), 16.6° (200/110) – the most prominent, in the region of 18.9–19.1º (014/203), 22.4º (210) belonging to the planes of the α- phase (card–00–054–1917) figure B1, appendix B) and at 2θ ≈ 16.7–16.8º (200/110) – the most prominent, 19.2–19.3º (201/111) and 22.3º (210), 24.2–24.7º (211) and 25.3–25.6º (202/112) belonging to the β- phase planes (card–00–054–1916, figure B2, appendix B) [84–87, 107]. The 0.7% and 1 wt % rGO/GO fillers in the PLLA matrix led to the appearance of a small peak in the region 2θ ≈ 15.0–15.3º indicating possibly the presence of a new crystal structure and reorganization of the PLLA domains [61, 66, 88]. It should be noted that the studied samples of PLLA–GO/rGO composites show reflections at 2θ ≈ 9.9º (111) belonging to the crystalline phase GO [89] and reflections 2θ ≈ 26.5º (002) belonging to the crystalline phase rGO [90]. X–ray diffraction rGO/GO nanopowders are a witness, the process of delamination and intercalation of graphite, and the final formation of graphene. The characteristic graphite peak at 2θ ≈ 26° expands with a decrease in the number of layers and ultimately disappears for monolayer graphene [91].

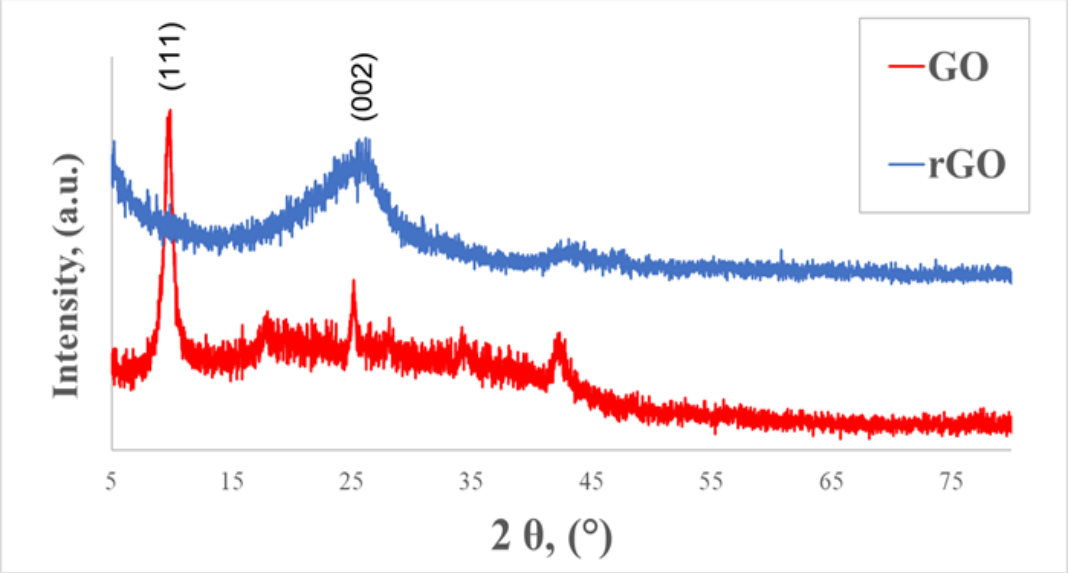


Figure 22 – The diffraction patterns of rGO and GO powders

It is known that crystallinity of the material is determined by the region of coherent X–ray scattering [90]. The addition of GO/rGO fillers in the PLLA matrix revelries the transformation from amorphous phase to crystalline phase ($2\theta \approx 16.6–16.7°$). PLLA–1%GO/rGO demonstrate the presence of (α- and β- phases). Hence, the above analysis proves that the addition of GO/rGO in the PLLA matrix affects the crystalline structure of PLLA matrix.

Conclusion

Since the structure of the solid state of PLLA consists of mainly amorphous phase, PLLA demonstrates a weak piezoelectric and mechanical performance that can limit their successful applications for regenerative bone tissue engineering [1, 3]. Therefore, to change the crystalline structure of PLLA, 2D rGO and GO nanofillers have been used in the present Master thesis. According to the literature analysis, the effect of doping rGO/GO on the structure of the PLLA, allowing to achieve a maximum piezoelectric response of the polymer, can be observed at a variety of rGO/GO concentrations up to 1.0 wt.%. In this regard, the following rGO/GO content has been added to PLLA: 0.2, 0.7 and 1.0 wt.%. Thus, a comprehensive study of GO/rGO impact on the morphology, crystalline and molecular structures of biodegradable PLLA–based scaffolds has been performed in the present work. The analysis of results has allowed to obtain the following main conclusions:

1. A defect–free fibrous nonwoven 3D scaffolds based on PLLA and doping 0.2%, 0.7%, 1% GO/rGO can be successfully fabricated via electrospinning.

2. The addition of GO/rGO led to decreasing the fiber diameter from (1.64±0.38) μm for PLLA to (1.02±0.40) μm for PLLA–1.0wt.% rGO and to (1.12±0.25) μm for PLLA–1.0wt.%GO.

3. Due to the annealing of PLLA scaffolds at 160°C for 2 h, fibers morphology became wavy and molecular structure transformed into the α-phase.

4. Analysis of TGA results revealed that the addition of GO/rGO fillers with the concentration up to 1 wt.% does not affect the thermal stability of the PLLA polymer scaffolds. TGA resulted in the following glass transition and melting temperature for PLLA scaffolds: T_g = (62.5±0.25) °C and T_m = (177.4±0.2) °C. In turn, DSC analysis revealed the crystallinity of (47.1 ± 0.9) % for PLLA scaffolds, which slightly decreased up to (42.8 ± 0.15) % after doping rGO/GO due to the reduced fiber diameter.

5. The addition of GO/rGO at 0.7 wt.% and 1.0 wt.% affected the crystalline structure of PLLA, since the presence of a peak corresponding to α- and β- phases was

observed in XRD patterns.

6. FTIR spectra of PLLA scaffolds with 0.7 wt.% rGO/GO show clear α- (912 cm^{-1}) and β-phase (921 cm^{-1}) peaks compared to other rGO/GO content. Thus, the addition of 0.7 wt.% rGO/GO induced a higher phase changes in PLLA.

Hence, the addition of 0.7 wt.% and 1.0 wt.% GO/rGO fillers in the 3D PLLA scaffolds demonstrates the effect on the crystalline structure of PLLA scaffolds. In turn, a clear presence of the β-phase in case of the 0.7 wt.% rGO addition into PLLA allows to expect a pronounced piezoelectric response for tension and compression at the same time. Thus, the elaborated hybrid biocomposites in the present study has a great potential for regenerative medicine.

The piezoelectric properties of these rGO/GO–PLLA scaffolds will be study in the future

Chapter 4. Financial management

4.1. Financial management, resource efficiency and resource saving

The purpose of this section discusses the issues of competitiveness, resource efficiency and resource saving, as well as financial costs regarding the object of study of Master's thesis. Competitiveness analysis is carried out for this purpose. SWOT analysis helps to identify strengths, weaknesses, opportunities and threats associated with the project, and give an idea of working with them in each particular case. For the development of the project requires funds that go to the salaries of project participants and the necessary equipment, a complete list is given in the relevant section. The calculation of the resource efficiency indicator helps to make a final assessment of the technical decision on individual criteria and in general.

4.1.1. Competitiveness analysis of technical solutions

In order to find sources of financing for the project, it is necessary, first, to determine the commercial value of the work. Analysis of competitive technical and methodical solutions in terms of resource efficiency and resource saving allows to evaluate the comparative effectiveness of scientific development. This analysis is advisable to carry out using an evaluation card. First of all, it is necessary to analyze possible technical solutions and choose the best one based on the considered technical and economic criteria Evaluation map analysis presented in table1.

Biodegradable hybrid electrospun fibers based on piezoelectric poly(L-lactic acid) and GO/rGO nanofillers is evaluated for each indicator on a five-point scale, where 1 is the weakest position and 5 is the strongest. The weights of indicators in the amount should be 1. Analysis of competitive technical solutions is determined by the formula:

$$C = \sum w_i \cdot p_i$$

C - the competitiveness of research or a competitor;

Wi– criterion weight;

Pi – point of it criteria.

Morphology, structure and piezoelectric response study of biodegradable hybrid

electrospun fibers based on piezoelectric poly(L–lactic acid) and GO/rGO nanofillers for regenerative tissue as a main methodic in this master thesis.

Moreover using 2 modules (ultrasound shaker for solution preparation and, Electrospinning technique for scaffolds fabrications) experiments was created (P_f). Competitors usually implement the following methods with some analysis: Optical Microscope, Scanning Electron Microscope (P_{i1}), FTIR, DSC, TGA, XRD, Piezo coefficient (P_{i2}).

Table 1. Evaluation card for comparison of competitive technical solutions.

Evaluation criteria	Criterion weight	Points			Competitiveness Taking into account weight coefficients		
		P_f	P_{i1}	P_{i2}	C_f	C_{i1}	C_{i2}
1	2	3	4	5	6	7	8
Technical criteria for evaluating resource efficiency							
1. Energy efficiency	0.05	2	5	4	0.1	0.25	0.2
2. Reliability	0.2	4	3	4	0.8	0.6	0.8
3. Safety	0.15	5	5	5	0.75	0.75	0.75
4. Functional capacity	0.1	4	2	3	0.4	0.2	0.3
5. Measurements accuracy	0.2	5	3	4	1	0.6	0.8
Economic criteria for performance evaluation							
1. Development cost	0.05	3	4	2	0.15	0.2	0.1
2. Scientific developments market penetration rate	0.15	5	3	5	0.75	0.45	0.75
3. Methodology perspectives	0.1	5	3	4	0.5	0.3	0.4
Total	1	33	28	31	4.45	3.65	4.1

As we can see after competitiveness analysis the highest competitiveness points is for our SEM, XRD(C_{if}) methodology and equal 4.45 in total, meanwhile for ,DSC (C_{i1}) total points equal 3.65 and TGA, FTIR (C_{i2}) equal 4.1. Most of the points XRD methodology received for functionality, measurement accuracy, methodology

perspective and scientific developments market penetration rate.

However, despite the fact that our methodology bypasses the other two in total, there are some problems with energy consumption and the cost of equipment and operation, which is a little high. In terms of cost and energy efficiency, it is most acceptable to use the XRD or SEM methods.

4.1.2.SWOT analysis

Complex analysis solution with the greatest competitiveness is carried out with the method of the SWOT analysis: Strengths, Weaknesses, Opportunities and Threats. The analysis has several stages. The first stage consists of describing the strengths and weaknesses of the project, identifying opportunities and threats to the project that have emerged or may appear in its external environment.

The second stage consists of identifying the compatibility of the strengths and weaknesses of the project with the external environmental conditions. This compatibility or incompatibility should help to identify what strategic changes are needed.

Table 2. SWOT analysis

	Strengths: S1. The results obtained with this XRD methodology are quite accurate. S2. The functionality of this technique allows to compare the results obtained using already known and new techniques. S4. DSC,FTIR methodology permit to obtain more in-depth information about the process of polymer crystallization S5. The equipment used for the XRD methodology is not narrowly targeted and can be used for other studies and experiments.	**Weaknesses:** W1. High cost of equipment. W2. It takes a lot of time to synthesize and prepare the necessary reagents, set up the equipment and process the results. W3. Equipment are constantly used by other researchers and engineers.
Opportunities: O1. Presence of well-	**Strengths:** *Using electrospinning*	*The equipment, reagents and materials that we use in*

educated researcher and teachers within the university facilities. O2. Fabricating two different materials as single composites material O3. Possibilities of studying new phases of crystallizations O4. Possibility of heating and cooling the sample.	*technique, it is possible to fabricate – many different regimes of fibers from Micro to Nano, Porous to more dense fibers morphology.* *By using ultrasound shaker , we can dissolve the different concentrations of PLLA powder, into GO/rGO and form solvent for Electrospinning* *SEM images relive the morphology and induced of GO/rGO on PLLA scaffolds.*	*experiments are a little expensive and the experiments themselves with their description take a lot of time. However, they help to obtain very precise and reliable data that can be reproduced in any other laboratory in the world with the same or similar equipment using our methodology.* *Equipment is constantly used by other researchers. Therefore, to reduce costs and lead time it is necessary to schedule our time of usage and to obtain as many experimental data as possible while the equipment is at our disposal.* *Since the materials are mainly custom-made or synthesized in Germany, China, Russia and then brought to Tomsk, it is necessary to plan what and how many specific materials we need, and then make an order in advance.*
Threats: T1. Since our methodology takes a long time, other scientists can get other results, process them and publish faster than we do. T2. The equipment used in the Ultrasound shaker and Optical Micro-scope techniques is not so expensive. T3. After the publication of our results, disputes may arise with other scientific researchers who use other methods.	*The technique that we use requires a lot of time to get good and accurate results However, we can speed up the process of describing and interpreting experimental data, as well as the process of preparing material for publication in scientific journals. can be used in Tissue engineering and Regenerative Medicine ,and Biosensors.*	*Speaking about the weaknesses and threats to our research, time is the determining factor. And defects in the scaffolds, which signifiently reduce the quality of the analysis.* *Solution viscosity plays a major role in the Electrospinning ,process, Applied voltage and distance, feed speed determines the fiber size.*

4.1.3 Project Initiation

The initiation process group consists of processes that are performed to define a new project or a new phase of an existing one. In the initiation processes, the initial purpose and content are determined and the initial financial resources are fixed. The internal and external stakeholders of the project who will interact and influence the

overall result of the research project are determined.

Table 3. Stakeholders of the project

Project stakeholders	Stakeholder expectations
RSCBT TPU	The results obtained from the hybrid piezo-scaffolds are used and taken into account in the scientific community for publications and for the therapeutic regenerative medicine.

Table 4. Purpose and results of the project

Purpose of project:	Comprehensive study of biodegradable hybrid electrospun fibers based on piezoelectric poly(l-lactic acid) and GO/rGO nanofillers for regenerative tissue engineering:
Expected results of the project:	The results show the addition of GO/rGO greatly influences the crystalline structure of PLLA.
Criteria for acceptance of the project result:	Results are repeatable and interpretable.
Requirements for the project result:	PLLA-Amorphous phase reduced when it is Annealed, and it demonstrates the presence of crystallinity phase
	PLLA- α, β phase crystallizations is obtained The results are interpreted, processed and verified.

4.1.4 The organizational structure of the project

It is necessary to solve some questions: who will be part of the working group of this project, determine the role of each participant in this project, and prescribe the functions of the participants and their number of labor hours in the project

Table 5. Structure of the project

№	Participant	Role in the project	Functions	Labor time, hours (working days (from table 7) × 6)
1	Jekhan	Researcher/ engineer	Experimentation, Data processing, Papers writing, Report writing	1800

2	Roman A. Surmenev	Head of project	Verification of work evaluating the results, Papers checking Report checking	456

4.1.5 Project limitations

Project limitations are all factors that can be as a restriction on the degree of freedom of the project team members.

Table 6. Project limitations

Factors	Limitations / Assumptions
3.1. Project's budget	300000 rubs
3.1.1. Source of financing	RSCBT TPU
3.2. Project timeline:	
3.2.1. Date of approval of plan of project	27.01.2019
3.2.2. Completion date	25.03.2020

4.1.6 Project Schedule

As part of planning a science project, you need to build a project timeline and a Gantt chart.

Table 7. Project Schedule

Job title	Duration, working days	Start date	Date of completion	Participants
Literature review /Research planning	41	3.01.2019	8.03.2019	Jekhan
Data processing of experimental results	240	17.05.2019	29.3.2020	Roman A. Surmenev / Jekhan
Experiments (PLLA-GO/rGO)	240	17.05.2020	29.03.2020	Jekhan
Results discussion	31	2.03.2020	7.04.2020	Roman A. Surmenev / Jekhan
Consultation with colleges	31	2.03.2020	7.04.2020	Roman A. Surmenev
Report writing	20	9.04.2020	29.04.2020	Jekhan

Financial management	12	13.04.2020	25.04.2020	Jekhan
Social responsibility	15	27.04.2020	16.05.2020	Jekhan
Report checking and corrections	13	10.05.2020	23.05.2020	Roman A. Surmenev

Total duration of working time – 367day

4.1.7 Gantt chart

A Gantt chart is a type of bar chart that illustrates a project schedule. This chart lists the tasks to be performed on the vertical axis, and time intervals on the horizontal axis. The width of the horizontal bars in the graph shows the duration of each activity.

Table 8. A Gantt chart

s.no	Activities	Participants	T_c days	Duration of Project										
				2019						2020				
				JAN-MAR	MAR-MAY	MAY-JULY	JULY-SEP	SEP-NOV	NOV-DEC	JAN	FEB	MAR	APR	MAY
1	Literature review/ research planning	Roman A. Surmenev / Jekhan	41											
2	Data processing of experimental results	Roman A. Surmenev / Jekhan	240											
3	Results discussion	Roman A. Surmenev / Jekhan	31											
4	Report writing	Jekhan	20											
5	Financial management	Jekhan	12											
6	Social responsibility	Jekhan	15											
7	Report checking and corrections	Roman A. Surmenev/	13											

Roman A. Surmenev.

Jekhan

4.1.8 Scientific and technical research budget

The amount of costs associated with the implementation of this work is the basis for the formation of the project budget. This budget will be presented as the lower limit of project costs when forming a contract with the customer.

To form the final cost value, all calculated costs for individual items related to the manager and the student are summed.

In the process of budgeting, the following grouping of costs by items is used:

- Material costs of scientific and technical research;
- costs of special equipment for scientific work (Depreciation of equipment used for design);
- basic salary;
- additional salary;
- labor tax;
- Overhead.

4.1.9 Calculation of material costs

The calculation of material costs is carried out according to the formula:

$$C_m = (1 + k_T) \cdot \sum_{i=1}^{m} P_i \cdot N_{consi},$$

where m – the number of types of material resources consumed in the performance of scientific research;

N_{consi} – the amount of material resources of the i-th species planned to be used when performing scientific research (units, kg, m, m^2, etc.);

P_i – the acquisition price of a unit of the i-th type of material resources consumed (rub. /units rub. /kg, rub. /m, rub. /m^2, etc.);

k_T – coefficient taking into account transportation costs.

Prices for material resources can be set according to data posted on relevant websites on the Internet by manufacturers (or supplier organizations).

Table 9. Material costs

Name	Unit	Amount	Price per unit rubs.	Material costs, rub.
PLLA –powder	50g	1	156000	79000
GO-powder	25g	1	50000	21500
rGO-powder	30 g	1	37000	8400
Chloroform, Acetone	500ml	5	17500	58500
Total				167400

4.1.10 Costs of special equipment

This point includes the costs associated with the acquirement of special equipment (instruments, stands, devices and mechanisms) necessary to carry out work on a specific topic.

Table 10. Costs of special equipment and software

№	equipment	Quantity of equipment	Price per unit, rub.	Total cost of equipment, rub.
1.	Ultrasound mixer	1	6000	6000
2.	Electrospining device	1	100000	100000
3	Needle, syring, solution delivery tubes,	50	1000	50000
4	Total			150600

Calculation of the depreciation

If you use available equipment, then you need to calculate depreciation:

$$A = \frac{C_{\text{перв}} * \text{H}_\text{a}}{100}$$

A - Annual amount of depreciation;

$C_{перв}$ - initial cost of the equipment;

$\text{H}_\text{a} = \frac{100}{\text{T}_{\text{сл}}}$ - rate of depreciation;

$T_{сл}$ - life expectancy.

№	equipment identification	Quantity of equipment	Total cost of equipment, rub.	Life expectancy, year	Cost of the project, rub.
1.	Optical Microscope	1	92000	10	92000
2.	Electrospinning	1	100000	10	100000
3	SEM	1	1000000	50	1000000
	FTIR	1	500000	50	500000
3.	DSC	1	550000	50	550000
4	TGA	1	550000	50	5500000
5.	XRD	1	127000	50	127000

Total for the title "Costs of special equipment" – 2416800 rubles.

4.1.11 Basic salary

This point includes the basic salary of participants directly involved in the implementation of work on this research. The value of salary costs is determined based on the labor intensity of the work performed and the current salary system

The basic salary (S_b) is calculated according to the formula:

$$S_b = S_d \cdot T_w, \tag{3.3}$$

where S_b – basic salary per participant;

T_w – the duration of the work performed by the scientific and technical worker, working days;

S_d - the average daily salary of a participant, rub.

The average daily salary is calculated by the formula:

$$S_d = \frac{S_m \cdot M}{F_v}, \tag{3.4}$$

where S_m – monthly salary of an participant, rub.;

M – the number of months of work without leave during the year:

at holiday in 48 days, M = 11.2 months, 6 day per week;

F_v – Valid annual fund of working time of scientific and technical personnel (244 days).

Table 11. The valid annual fund of working time

Working time indicators	Days
Calendar number of days	365
The number of non-working days - weekend - holidays	 52 14
Loss of working time - vacation - isolation period - sick absence	 48 7
The valid annual fund of working time (F_d)	244

Monthly salary is calculated by formula:

$$S_{month} = S_{base} .(K_{premium} + K_{bonus}).K_{reg} \quad (x)$$

where S_{base} – base salary, rubles;

$k_{premium}$ – premium rate;

k_{bonus} – bonus rate;

k_{reg} – regional rate.

Table 12. Calculation of the base salaries

Performers	S_{base}, rubles	$k_{premium}$	k_{bonus}	k_{reg}	S_{month}, rub.	W_d, rub.	T_p, work days	W_{base}, rub.
Jekhan	17 890	1228	84	103152	26746-	1228-	310	380680
Roman A. Surmenev	49141				73466	3372	76	256272

Total for the title "Basic salary" -636952 rubles.

4.1.12 Additional salary

This point includes the amount of payments stipulated by the legislation on labor, for example, payment of regular and additional holidays; payment of time associated with state and public duties; payment for work experience, etc.

Additional salaries are calculated on the basis of 10-15% of the base salary of workers:

$$W_{add} = k_{extra} \cdot W_{base}, \quad \text{(x)}$$

where W_{add} – additional salary, rubles;

k_{extra} – additional salary coefficient (10%);

W_{base} – base salary, rubles.

W_{add} (A.M.S.Jekhan) = 38068 rubles.

W_{add} (Roman A. Surmenev) = 25627 rubles.

Total for the title "Additional salary" –63695 rubles.

4.1.13 Labor tax

Tax to extra-budgetary funds are compulsory according to the norms established by the legislation of the Russian Federation to the state social insurance (SIF), pension fund (PF) and medical insurance (FCMIF) from the costs of workers.

Payment to extra-budgetary funds is determined of the formula:

$$P_{social} = k_b \cdot (W_{base} + W_{add}) \quad \text{(x)}$$

where k_b – coefficient of deductions for labor tax.

In accordance with the Federal law of July 24, 2009 No. 212-FL, the amount of insurance contributions is set at 30%. Institutions conducting educational and scientific activities have rate - 27.1%.

Table 13. Labor tax

	Project leader (Roman A. Surmenev)	**Engineer (Jekhan)**
Coefficient of deductions	0.271	
Salary (basic and additional), rubles	281899	418748
Labor tax, rubles	76395	113480

Total for the title "Labor tax" –189875 rubles.

4.1.14 Overhead costs

Overhead costs include other management and maintenance costs that can be allocated directly to the project. In addition, this includes expenses for the maintenance, operation and repair of equipment, production tools and equipment, buildings, structures, etc.

Overhead costs account from 30% to 90% of the amount of base and additional salary of employees.

Overhead is calculated according to the formula:

$$C_{ov} = k_{ov} \cdot (W_{base} + W_{add}) \quad \text{(x)}$$

where k_{ov} – overhead rate.

Table 14. Overhead

	Project leader (Roman A. Surmenev**)**	**Engineer** (Jekhan)
Overhead rate	0.3	
Salary, rubles	281899	418748
Overhead, rubles	84570	125624

Total for the title "Overhead costs" –210194 rubles

4.1.15 Other direct costs

Energy costs for equipments are calculated by the formula:

$$C = P_{el} \cdot P \cdot F_{eq},$$

where

P_{el} − power rates (5.8 rubles per 1 kWh);

P − power of equipment, kW;

F_{eq} − equipment usage time, hours.

C (optical microscope) = 418 rubles;

C (Electrospining) = 626 rubles;

C (XRD) = 835 rubles;

C (FTIR) = 209 rubles;

C (DSC,TGA) = 950 rubles.

Total for the title "Other direct costs" – 3038 rubles.

Formation of budget costs

The calculated cost of research is the basis for budgeting project costs.Determining the budget for the scientific research is given in the table 16

Table 16. Items expenses grouping

Name	Cost, rubles
1. Material costs	150600
2. Equipment costs	2416800
3. Basic salary	636952
4. Additional salary	63695
5. Labor tax	189875
6. Overhead	210194
7. Other direct costs	3038
Total planned costs	**3671154**

4.1.16 Conclusion

Thus, in this section was developed stages for design and create competitive development that meet the requirements in the field of resource efficiency and resource saving.

These stages include:

- development of a common economic project idea, formation of a project concept;
- organization of work on a research project;
- identification of possible research alternatives;
- research planning;
- assessing the commercial potential and prospects of scientific research from the standpoint of resource efficiency and resource saving;
- Determination of resource (resource saving), financial, budget, social and economic efficiency of the project.

Comprehensive study, morphology, structure and piezoelectric response of biodegradable hybrid electrospun fibers besed on piezoelectric poly(l-lactic acid) and GO/rGO nanofillers for regenerative tissue engineering: The obtained analysis results proves, the addition of fillers affects the the Crystaline structure of PLLA scaffolds allows to expect a pronounced piezoelectric response for tension and compression at the same time.

Chapter 5. Social responsibilities

5.1.Introduction

A biodegradable piezo- scaffolds based on PLLA-GO/rGO is fabricated via electrospinning device and study its structural and morphology ,influences of GO/rGO nanofillers in the PLLA matrix for regenerative tissue.A series of experiments undergone to fabricate defects free samples made in the course of work. The experiments, technical calculations, design and fabrication of scaffolds were carried out in TPU laboratory, 3 building, 108 room. The purpose of this section is to analyze and evaluate harmful and hazardous labor factors that may affect project development personnel. The development of protective measures against these factors, the assessment of working conditions. Also in this section, issues related to safety, fire prevention and environmental protection, recommendations for creating optimal working conditions are considered. During the development and operation of the designed solution, the following harmful factors should be taken into account: fibrous scaffolds are obtained by Electrospinning device. This type of device is highly danger due to its high voltage. When conducting experiments, special measures are taken to ensure the safety. During the solution preparation special care is given, due to handling of the harmful chemicals.

When working with the Electrospinning device, one should be guided by the following document

GOST 12.1.038–82 SSBT Electrical safety Maxium permissible levels of contact voltage and currents

Lab coats and gloves are mandatory for conducting the experiments to avoid any chemical hazards.

5.1.1 Special legal norms of labor legislation

In according to [1]

- The normal duration of working time cannot exceed 40 hours per week;
- During the working day, the employee should be given a break for rest and

meals lasting no more than two hours and at least 30 minutes, which is not included in working hours;

- All employees are given days off (weekly continuous rest). With a five-day working week, employees are given two days off per week; with a six-day working week, one day off;
- Annual vacations with retention of the place of work and average wage should be paid to employees;
- Annual paid leave is granted to employees lasting 28 calendar days, in accordance with [2].
- In accordance with [3], persons over 18 years old, who have undergone special training. Who training in the prescribed manner to work with a chemical product and certification to the labor protection group during a work on electrical installations with the appropriate equipment is allowed to work with Electrospinning device.

In accordance with Art. 221 - 225 of the Labor Code of the Russian Federation, at work with harmful and (or) hazardous working conditions, as well as at work performed in special temperature conditions or related to pollution, employees are given free compulsory certification or declaration of compliance with special clothing, special shoes and other means personal protection, as well as washing and (or) neutralizing agents in accordance with the model rules, which are established in the manner determined by the Government of the Russian Federation. It is also envisaged to undergo a medical examination for workers who perform work in conditions with hazardous and (or) harmful production factors, which is specified in article 213 of the Labor Code of the Russian Federation. Moreover, and in employment, and in the process. In the order of the Ministry of Health and Social Development of the Russian Federation of 12.04.2011 No. 302n. Specified procedure for conducting a medical examination. The requirements of this document provide that a medical examination should be carried out once a year, or twice a year. It depends on the type of activity of the worker in production, as well as on the presence of

specific harmful factors.

According to Part 6 of Article 213 of the Labor Code of the Russian Federation, workers engaged in certain types of activities, including those associated with sources of increased danger (with the influence of harmful substances and adverse production factors), as well as working in conditions of increased danger, undergo an obligatory psychiatric examination at least once every five years in the manner established by the federal executive body authorized by the Government of the Russian Federation

5.1.2. Organizational measures in the layout of the working area

When performing research for this project, the work is doing by the operator in a sitting position. In accordance with [5]:

The workplace should ensure the performance of labor operations within the reach of the motor field. The performance of labor operations “often” and “very often” must be ensured within the zone of easy reach and the optimal zone of the motor field.

The design of production equipment and the workplace should ensure the optimal position of the worker, which is achieved by regulation:

1. Height of the working surface, seat and leg space;
2. The height of the seat and footrest (with unregulated height of the working surface).

The optimal working position for working man of lower growth is achieved by increasing the height of the working seat and footrest by an amount equal to the difference between the height of the working surface for a worker with a height of 1800 mm and the height of the working surface that is optimal for the growth of this worker. Requirements for the height of the workplace are shown in table 1.

Table 1 – A height of a workplace surface with various types of work

Name of works	A height of a workplace surface, mm		
	Women	Men	Women and men
Delicate work	700	750	725
Light work	630	680	655

Workplaces should be organized in such a way as to safely exclude the experiments (solution formation and samples preparation) in a specially designated areas that comply with fire safety requirements and have the necessary fire prevention and fire protection equipment [3].

5.1.3.Industrial safety

Harmful and dangerous factors are analyzed that may occur during the development or operation of the device under development in this section
GOST 12.0.003-201 is used to select factors. A list of hazardous and harmful factors is presented, which are typical for the designed production environment in the form of a table

Table 2 – Hazardous and harmful factors in the development of a method

Source of factor, name of type of work	Factor		Regulations
	Harmful	Dangerous	
Experiment 1-Preparation of the solutions; 2-apparatus setup(ES) 3-fabrication of the scaffolds 4-study and analysis of the obtained samples	1-Deviation of microclimate indicator 2-Insufficient illumination of the working area 3-High voltage 4-Chemical hazards	1-Fire 2- Electrical current	1. GOST 31581-2013; 2- GOST 12.1.038–82 SSBT Electrical safety 3- GOST 12.1.030-81 4- GOST 12.1.005-88; 5- GN 2.1.6.3492-17; 6- SP 60.13330.2012

Identified harmful and dangerous factors are discussed in more detail below. Each factor is considered in sequence: the source of the factor; reduction of permissible norms with the required dimension; safety products

5.2. Analysis of hazardous and harmful industrial factors

5.2.1 Requirements for safe operation of electrospinning device

The following special rules must be observed when operating the Electrospinning device: systematically monitor and maintain all safety devices

(screen, panels, doors, locks, alarms) in good condition. The shutdown procedure must be strictly observed, at the end of which remove the key of the main switch from the lock.

Always the safety lock should be turned on during the experiment the electrode should be properly connected. The ground wire should be properly grounded.

5.2.2. Increased noise level

During electrospinning, a abundant noise occurs, At the workplace, noise occurs when the lamps of the pulsed heating, the ventilation of a personal computer and when exposed to external factors.

Noise adversely affects the human body, causes mental and physiological disorders, hearing loss, performance, creates the prerequisites for common and occupational diseases and industrial injuries, as well as weakening of memory, attention, violation of blood pressure and heart rate.

Noise levels should not exceed the values specified in GOST 12.1.003 - 2014, and they should be checked at least twice a year. The main characteristic of noise is the maximum permissible noise level (RC). The maximum permissible level (noise level) of a noise is the level of a factor that, when working daily (except weekends), but not more than 40 hours a week during the entire working period, should not cause diseases or deviations in health, which are detected by modern research methods process of work or in the remote periods of life of the present and subsequent generations. Compliance with the noise level of the remote control does not preclude health problems in hypersensitive individuals.

According to GOST 12.1.003 - 2014, the noise parameters are normalized and during the work the noise level should not exceed 82 dB;

At values above the permissible level, it is necessary to provide for personal protective equipment and collective protection devices against noise.

Collective protection:

- elimination of the causes of noise or its significant weakening in the source of education;
- isolation of noise sources from the environment (use of silencers, screens, sound-absorbing building materials);
- the use of tools that reduce noise and vibration in the way of their distribution;
- The use of protective clothing and hearing aids: headphones, earplugs, antiphons.

5.3. Analysis of harmful and dangerous factors that may arise in the laboratory during research

5.3.1. Climate deviation

Favorable conditions for the workplace microclimate must be created while working in the laboratory. Prolonged exposure of a person to adverse weather conditions can dramatically worsen his well-being, reduce labor productivity and lead to diseases. The microclimate is determined by combinations of temperature, humidity, air velocity and thermal radiation acting on the human body. High air temperature contributes to the rapid fatigue of the worker, and can lead to overheating of the body, cause a violation of thermoregulation, impairment of well-being, decreased attention, heat stroke, increased stress on the heart. Low air temperature can cause local or general hypothermia, cause colds, and lead to diseases of the peripheral nervous system (radiculitis, bronchitis, rheumatism). Low humidity can cause the mucous membranes of the respiratory tract to dry out. Air mobility effectively contributes to the heat transfer of the human body and is positively manifested at high temperatures and negatively at low. optimal and permissible microclimate

Table 3 – Optimum microclimate indicators at workplaces of industrial premises

Period of the year	Category of work on the level of energy consumption,W	Air temp. C.	Relative humidity, %	Air velocity, m/s
Cold	IIa(175-232)	19-21	60-40	0.2
Warm	IIa(175-232)	20-22	60-40	0.2

Table 4 – Permissible microclimate indicators

Period of the year	Air temperature, C		Relative humidity, %	Air velocity, m/s	
	Upper bound	Lower bound		Upper bound	Lower bound
Cold	23-24	15-17	40-60	0.1	0.2
Warm	27-29	17-18	40-60	0.1	0.3

5.3.2. Electrical safety

Electrical safety is a system of organizational and technical measures aimed at protecting people from the harmful and dangerous effects of electric current. There is a danger of electric shock in all cases where electrical installations and equipment are used. Electrical installations are classified by voltage - with a rated voltage of up to 1000 V (rooms without increased danger), up to 1000 V with the presence of an aggressive environment (rooms with increased danger) and over 1000 V (rooms especially dangerous) (according to the Rules for the Installation of Electrical Installations).

To ensure safe operation, it is necessary to exclude possible sources of electric shock:

1. Accidental contact with live parts under voltage;
2. The appearance of voltage on the mechanical parts of electrical equipment (cases, covers, etc.) due to insulation damage or other reasons;
3. The occurrence of stress on the ground or supporting surface.

According to the degree of danger of electric shock, this laboratory belongs to rooms without increased danger, it is a dry room without increased dusting, the air temperature is normal, the floor is covered with insulating material.

All equipment and devices are in place and have protective grounding with a resistance of not more than 4 Ohms [9]. All employees undergo initial electrical safety training.

It is necessary to check the serviceability of conductive wires before starting work. It is forbidden to use wires with damaged insulation or without insulation, as well as wires that are not equipped with plugs or soldered terminals, to connect electrical appliances. Instruments must be kept clean. It is necessary to disconnect the equipment from the network at the end of work.

Electric shock may occur because of careless operations with connecting wires. In addition, a short circuit can occur when current-carrying parts close on the device about absence of nulling or grounding and cause the electric shock.

Table 5 – Permissible levels of effective touch voltage and currents

Mode	Current type					
	AC, 50 Hz			DC		
	U, V	I, mA	Duration, min	UV	I, mA	Duration, min
Normal	2	0,3	<10	8	1	<10

First aid to the victim should consist in immediately disconnecting the current that caused the injury, disconnecting (in rubber gloves) the victim from the leads and calling the doctor. If the victim is conscious, but before that he was swooning or has been under current for a long time, he needs to ensure peace before the doctor arrives. If the victim lost consciousness, but breathing persists, it is necessary to put it comfortably, evenly, unfasten tight clothing, create an influx of fresh air, remove unnecessary people from the room, breathe ammonia, spray with water, rub and warm the body. It is necessary to apply artificial respiration with convulsive and rare breathing. In the absence of signs of life (lack of pulse and breathing), the victim cannot be considered dead. It is necessary immediately, without wasting time, before the arrival of the doctor to do artificial respiration.

5.3.3. Room illumination

Light sources with a color temperature of 2400 to 6800 K should be used for general and local lighting of rooms. The intensity of ultraviolet radiation in the wavelength range of 320-400 nm should not exceed 0.03 W/m [10].

The presence in the radiation spectrum of wavelengths less than 320 nm is not allowed.

For artificial lighting, energy-efficient light sources should be used, giving preference to equal power sources of light with the highest light output and service life, taking into account the requirements for color differentiation.

5.3.4. Fire hazard

When fabricating the scaffolds via electrospinning, an emergency situation of a fire nature may occur.

The fire that causes material damage. According to GOST 12.1.033 - 81, the concept of fire safety means the condition of an object in which the probability of occurrence and development of a fire and the exposure of people to dangerous factors of a fire is excluded with an established probability, and material values are also protected.

Fire safety involves ensuring the safety of people and preserving the material values of an enterprise at all stages of its life cycle. The main fire safety systems are fire prevention and fire protection systems, including organizational and technical measures.

According to the explosion and fire hazard of the premises are divided into categories A, B, B1-B4, G and D, and the building into categories A, B, C, G and D.

According to NPB 105-03, a laboratory is classified as category B - combustible and difficult combustible liquids, solid combustible and difficult combustible substances and materials, substances and materials that can only burn when interacting with water, oxygen or any other oxygen, provided that the rooms, in which it is located, are not classified as the most dangerous A or B.

According to the degree of fire resistance this room belongs to the 1st degree of fire resistance according to SNiP 2.01.02-85 (made of brick, which refers to

difficult-to-combustible materials).

The occurrence of a fire when working with electronic equipment may be due to both electrical and non-electrical reasons.

To localize or eliminate ignition at the initial stage, primary fire extinguishing agents are used. Primary fire extinguishing agents are usually used until the fire brigade arrives.

Fire extinguishers (OHVP-10) are used to extinguish the fires without the presence of electricity. Carbon dioxide (OU-2) and powder fire extinguishers are designed to extinguish electrical installations that are under voltage up to 1000V. To extinguish current-carrying parts and electrical installations, a portable powder fire extinguisher is used, for example OP-5

At least two portable fire extinguishers should be placed in public buildings and structures on each floor. Fire extinguishers should be located in prominent places in the vicinity of exits from the premises at a height not exceeding 1.35 m. Placing primary fire extinguishing equipment in corridors and passages should not impede the safe evacuation of people.

To prevent fire and explosion, it is necessary to provide for:

- special insulated rooms for the storage and spill of flammable liquids (flammable liquids), equipped with supply and exhaust ventilation in explosion-proof design - in accordance with GOST 12.4.021-75 and snip 2.04.05-86;
- special rooms (for storage in containers of dusty rosin), isolated from heating devices and heated equipment parts;
- primary fire extinguishing equipment at production sites (mobile carbon dioxide fire extinguishers GOST 9230-77, foam fire extinguishers TU 22-4720-80, sandbags,);
- The laboratory fully complies with fire safety requirements, namely, the presence of fire alarm, evacuation plan, shown in Figure 1, powder fire extinguishers with an attorney stamp, signs indicating the direction to the emergency exit.

Figure 1– Evacuation Plan

The TPU building in which the laboratory is located complies with fire safety requirements

5.3.5. Determination of air exchange in laboratory

Air exchange in public buildings is necessary to clean the air of harmful substances: to remove harmful substances (emitted harmful gases, vapors and dust), to remove water vapor and excess heat.

In residential and public buildings, carbon dioxide (CO_2) exhaled by people is a constant harmful emission. The required air exchange is determined by the amount of carbon dioxide exhaled by a person and by its permissible concentration. The amount of carbon dioxide, depending on the age of the person and the work performed, as well as the permissible concentration of carbon dioxide for different rooms. The carbon dioxide content in the air can be determined by the chemical composition of the air. However, given the increased carbon dioxide content in the atmosphere of settlements, the CO_2 content should be taken into account when calculating:

- for large cities (over 300 thousand inhabitants)– 0.5 l/m^3.

Determine the required rate of air exchange in a laboratory for three people, if the volume of the room is V=72 m^3. The amount of carbon dioxide exhaled by an adult with light work in an institution is 23 l/h. The maximum permissible concentration of carbon dioxide for institutions is 1.25 l/m^3. The required air exchange in the laboratory

is determined by the formula 1:

$$L = \frac{G*P}{Xv-Xn}$$

where L – air exchange required, m^3/h;

G – the amount of harmful substances released into the room air, g/h; P – number of people working in the laboratory;

x_v – maximum permissible concentration of harmfulness in the air of the working area of the room [7], mg/m^3;

x_n – the maximum possible concentration of the same harmfulness in the air of populated areas [12], mg/m^3.

The rate of air exchange (n), which shows how many times in one hour the air is completely replaced in the room, which is determined by the formula 2

(2)

$$N = \frac{L}{V_n}, h^{-1}$$

where V_n is the internal volume of the room, m^3.

According to [13], the permissible air exchange rate should be in the range from 3 to 10 h^{-1}.

Required air exchange in the laboratory, according to 1:

$$\frac{23 * 3}{1.25 - 0.5} = 92 \frac{m^3}{h}$$

The required air exchange rate is:

$$\frac{92}{72} = 1.27\ h^{-1}$$

Thus, the calculated consumed air exchange in the laboratory should be 92m^3/h.

5.4. Environmental safety

5.4.1. Analysis of the impact of the object of research on the environment

This subsection considers the environmental impact of the laboratory facility. The alleged sources of environmental pollution resulting from the implementation of

the solutions proposed in the are identified.

During operation of the facility, the main types of impacts of the designed facility are established:

- household waste;
- Industrial waste;
- Chemical waste.

Measures to reduce the intensity of environmental pollution is the creation of obstacles to the distribution and treatment of waste by various methods.
Utilization of household, Industrial and Chemical waste is the Main Event. Glass, metal waste, waste paper, as well as plastics are processed into secondary raw materials.

Class B industrial wastes (syringe needles, metal pieces and waste wires) are collected in separate disposable soft or hard packaging. The choice of packaging depends on the morphological composition of the waste.

To collect chemical waste of class B, disposable, non-punctureable, moisture-resistant containers with a lid (containers) must be used to ensure their sealing and exclude the possibility of spontaneous opening.

After filling the bag by no more than 3/4, the person responsible for the collection of waste in this unit fastens the bag or closes it using tag tags or other devices that prevent the discharge of Class B waste. Solid containers are closed with lids. Class B waste disposal outside the unit in open containers is not permitted. At the final packaging of class B waste to remove it from the unit (organization), disposable containers with class B waste are marked with the inscription "Waste.

Class B" with the name of the organization, unit, date and name of the person responsible for collecting the waste.Class B medical waste (gloves and containers) from units in closed disposable containers is placed in containers. Then they are moved to a waste management site or a temporary storage room for medical waste until the next transport of specialized organizations to the place of disinfection. Access by unauthorized persons to the temporary storage of medical waste is prohibited. [14].

During operation, the atmosphere is not polluted

5. Safety in emergency situation

5.5.1.Analysis of probable emergencies that may occur in the laboratory during research

In case of emergency, you must immediately call the fire department at number “01” from your business phone or “101” from your mobile phone.

The notification of civil defense alerts in the event of an emergency to the personnel of the facilities is carried out using voice information via broadcasting channels, radio broadcast networks and communication networks. On the territory of TPU they do not use, do not produce, do not process, do not store radioactive, fire hazardous, as well as explosive substances that create a real threat of an emergency source. As the most probable technological emergencies, the project considers:

- fire in the facility.

Fire hazards for humans include toxic combustion products, low oxygen concentration, open flames, smoke, and high air temperatures.

The following measures must be observed to prevent fire:

1. Reducing the determining size of the combustible medium;

2. Prevention of the formation of a combustible medium.

In case of overheating, short circuits, etc. possible ignition of electrical installations, wiring. To extinguish the fire, it is necessary to use special means, it is impossible to use water and other conductive substances. Therefore, the premises should be equipped with means for extinguishing electrical installations and electrical wiring under voltage

Safe Systems of Work

1. Know about space & equipment
    - Know your building:-
    - Exit routes
    - Areas of rescue assistance
    - Shelter in place locations

- Assembly location

2.Know your gear:-

- First aid supplies
- Emergency Procedures poster
- Keep a Go Bag (flashlight, emergency contacts, etc.)

3. Hearing about an emergency

- Siren
- Fire alarm
- Social media
- Alerted by a colleague
- Call from a friend
- Witness the event

4. Getting help:-

- For any type of emergency call police or administrator
- Give your name, address, and the nature of the emergency
- Stay on the line until you are told you may hang up
- Remain calm and answer questions as clearly as possible

5. Deciding what to do - evaluation

- Gather information Watch and listen for instructions
- Follow instructions
- Shelter in Place = Stay
- Evacuate = Go Sometimes you just have to use your best judgment

5.6 Conclusion

This section provided a description of social responsibility that accompanies the implementation of final qualifying work.

Industrial and environmental safety were described, various harmful and dangerous factors and methods of combating them were identified. In addition, a list

of measures to reduce the threat in the event of an emergency was given, and legal and organizational security issues were examined. On top of this, the most important points of the organizational arrangements for the design of the working area are presented

Acknowledgments

Throughout the writing of this thesis, I have received a great deal of support and assistance. I would first like to thank my supervisor **Dr. Roman A. Surmenev**, whose expertise was invaluable in the formulating of the research topic and methodology in particular.

I would especially grateful to thank **Mr Roman V. Chernozem** and **Mrs Julia R. Mukhortova**, for their valuable guidance throughout this project. I have learned not only the technical and academic skills, but also how to face the unexpected problems and to evaluate the importance of the research work.They guided me with the tools and the knowledge during the samples preparation in the lab for to successfull completion of my dissertation.

Special thanks to **Mr.Alexey Zinoviev** for helping and guiding me through the research work.

In addition, I would also like to thank my **family members in India** for giving me a chance to study abroad all the support and encouragement they have given to me are greatly appreciated

Appendix A. PLLA characteristic bands in IR spectra

Table A1. PLLA IR characteristic bands

Vibration	**Wavenumber, cm^{-1}**
Asymmetric C – H stretching vibration CH_3	2994
Asymmetric C – H bending vibration CH3	1357 1366 1382
Symmetric C – H stretching vibration CH3	2920 2850
Symmetric C – H bending vibration CH3	1450
C = O bending	1754 1268 1240
C – O – C stretching	1182
C – O stretching	1048
Amorphous	**955**
Crystalline, α’	923 – 925 916 - 918
Crystalline, α (C −C backbone stretching vibration CH3)	921
Crystalline, β	**908 - 912**
Crystalline, α	**871**
C – O – C bending vibration	860

Appendix B (α-/β- phase difraction pattern of PLLA matrix)

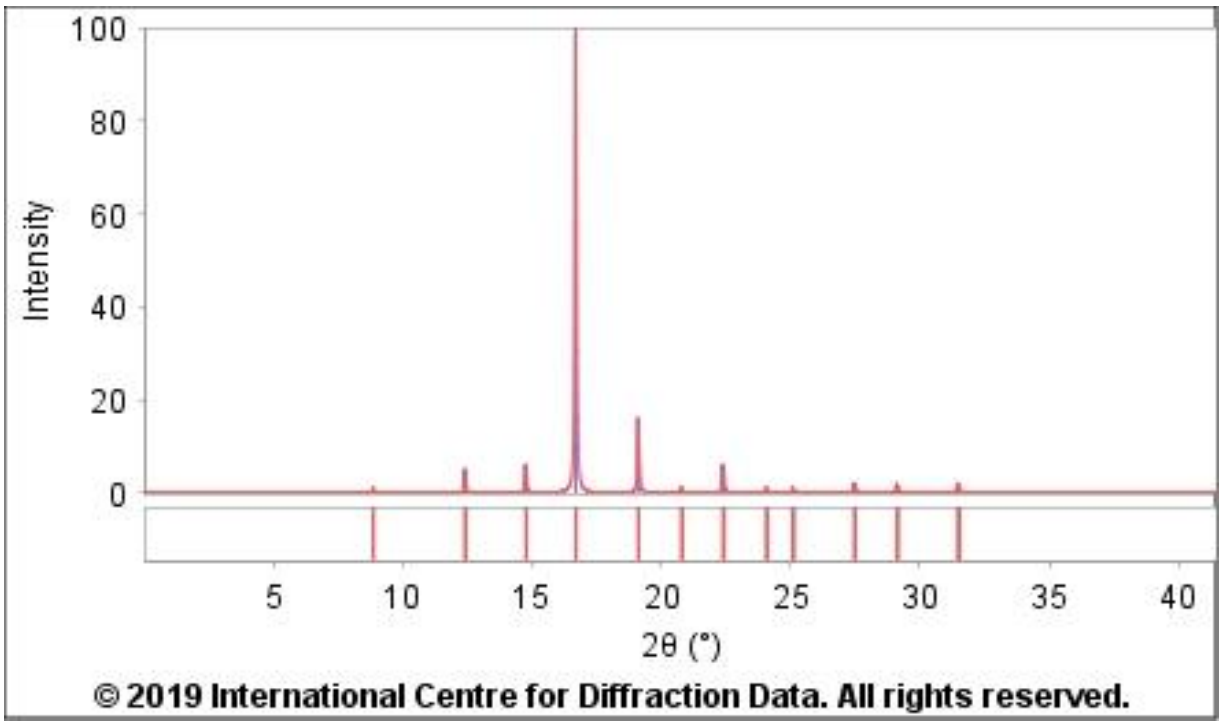


Figure B1 – PLLA α- phase planes – (card #54– 1917).

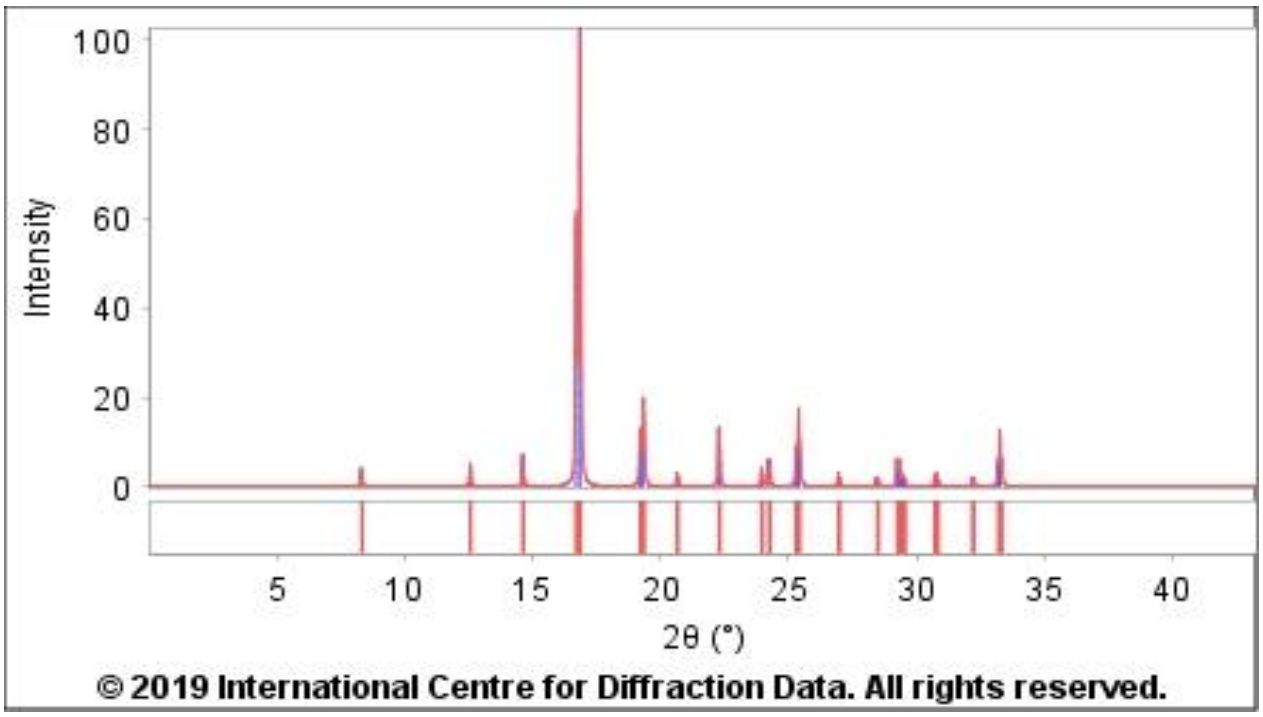


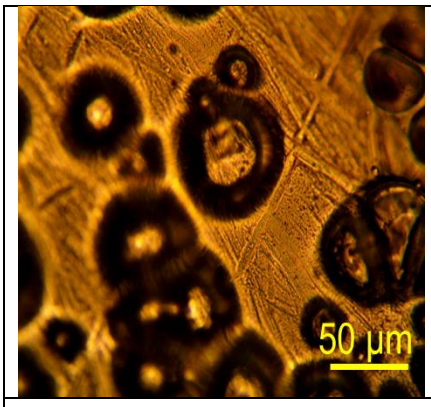
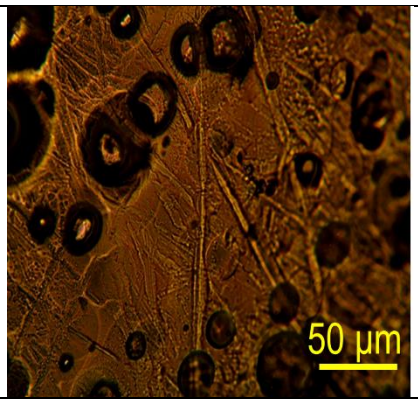
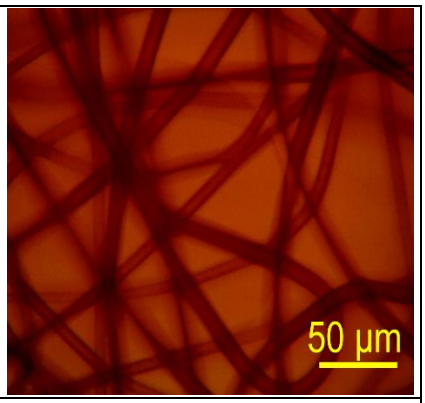
Figure B2 – PLLA β- phase planes (card #54–1916)

Appendix C. Influence of PLLA concentration in fiber formation

To form fibrous structure based on PLLA, firstly the influence of the polymer concentration has been studied. PLLA concentration in the solution varied from 4 to 10 wt.%. Before ES experiments, PLLA (M_w – 210,000 g/mol) was dissolved in chloroform. The ES was carried out at the following parameters: needle – 0.51 mm/G 27; feed speed – 0.3 ml/h; distance from needle – 10 cm; voltage – 3.6–4.7 kV.

The obtained optical images of the samples with different PLLA concentrations (4, 7 and 10 wt.%) in the polymer solution are presented in the Table 1. Optical microscopy demonstrates that samples with low concentration of PLLA (4–7 %) have the structure of the spheres. Meanwhile, the increase of the PLLA concentration in the solution up to 10 wt.% led to formation defect–free fibers.

Table C – Influence of PLLA concentration in fiber formation

		
PLLA **4%** – no fibers	PLLA **7%** – no fibers	PLLA **10%** – fibers dia. 7.75 (± 1.42) µm

Data obtained by dissolving PLLA in chloroform; needle – d=0.51 mm (G 27); feed speed – 0,3 ml/h; distance from needle – 10 cm; voltage – 3,6–4,7 kV.

Reference

1. Alam, M.K. Recent Advances in Microfluidic Technology for Manipulation and Analysis of Biological Cells (2007-2017)/ M.K. Alam, E. Koomson, H. Zou [et al.] // Anal. Chim. Acta. – 2018. – V.1044 – P. 29-65.
2. Ali, M. Controlling laser-induced jet formation for bioprinting mesenchymal stem cells with high viability and high resolution/ M. Ali, E. Pages, A. Ducom [et al] // Biofabrication. – 2014. – 6:045001
3. Alkayyali, T. Microfluidic and cross-linking methods for encapsulation of living cells and bacteria - A review / T. Alkayyali, T. Cameron, B. Haltli [et al.] // Anal. Chim. Acta. – 2019. – V. 1053. – P.1 – 21.
4. Alvarez, K., and Nakajima, H. Metallic Scaffolds for Bone Regeneration Materials. – 2009. – V.2. – P.790 – 832.
5. An, Y. Bone Marrow Mesenchymal Stem Cell Aggregate: An Optimal Cell Therapy for Full-Layer Cutaneous Wound Vascularization and Regeneration/ Y. An, W. Wei, H. Jing [et al.] // Sci. Rep. – 2015. – 5:17036.
6. Schultz, O. Emerging strategies of bone and joint repair / O. Schultz, M. Sittinger, T. Haeupl [et al.] // Arthritis Res. – 2000. – V. 2. P. 433 – 436.
7. Alsberg, E. Craniofacial Tissue Engineering /E. Alsberg 1, E.E. Hill, D.J. Mooney // Crit Rev Oral Biol Med. – 2001.–V. 12 (1). – P. 64–75.
8. Langer, R. Tissue engineering / R. Langer, J.P. Vacanti // Science. – 1993. – V. 260. – P. 920–926.
9. Lysaght, M.J. The Growth of Tissue Engineering / M.J. Lysaght, J. Reyes // Tissue Eng. – 2001. – V.7. – P. 485–493.
10. Loty, C. In vitro bone formation on a bone-like apatite layer prepared by a biomimetic process on a bioactive glass–ceramic / C. Loty, J-M. Sautier, H. Boulekbache [et al.] // Biomed Mater Res. – 2000. – V. 49. – P. 423–434.
11. Meyer U. Biological and biofisical principals in extracorporal bone tissue engenearing / U. Meyer, U. Joos, H.P. Wiesmann // Part I. Int J Oral Maxillofac Surg. – 2004. – V. 3. – P. 325–332.

12. Schliephake, H. Use of Cultivated Osteoprogenitor Cells to Increase Bone Formation in Segmental Mandibular Defects: An Experimental Pilot Study in Sheep / H Schliephake, J W Knebel, M Aufderheide, M Tauscher // Int J Oral Maxillofac Surg. – 2001. – V. 30. – P. 531–537.
13. Lang N.P. Guided tissue regeneration in jawbone defects prior to implant placement / N.P. Lang, C.H.F. Hämmerle, U. Brägger [et al.] // Clin Oral Implants Res. – 1994. – V. 5(2). – P. 92–97.
14. Buser, D. Lateral Ridge Augmentation Using Autografts and Barrier Membranes: A Clinical Study With 40 Partially Edentulous Patients/ D. Buser, K. Dula, H. P. Hirt, R. K. Schenk // JOral Maxillofac Surg. – 1996. – V.54. – P. 420–432.
15. Berglundh, T. Healing Around Implants Placed in Bone Defects Treated With Bio-Oss. An Experimental Study in the Dog / T. Berglundh, J. Lindhe // Clin Oral Implants Res. – 1997. – V. (8). – P. 117–124.
16. Hoang, N.T. Neovascularization in prefabricated flaps using a tissue expander and an implanted arteriovenous pedicle / N. T. Hoang, M. Kloeppel, R. Staudenmaier [et al.] // Microsurgery. – 2005. – V. 2. – P.213–219.
17. Top, H. Bone flap prefabrication: an experimental study in rabbits. Ann Plast Surg / H. Top, C. Aygit, A. Sarikaya et al [et al] //Ann PlastSurg. – 2005. – V. 54. – P. 428–434.
18. Fisher, J. Experimental comparison of bone revascularization by musculocutaneous and cutaneous flaps / J. Fisher, M.B. Wood // Plast Reconstr Surg. – 1987. – V. 79. – P. 81–90.
19. Findlay, M. Creating large amounts of tissue for reconstructive surgery—a porcine model / M. Findlay, J. Dolderer, J. Cooper-White [et al] // Aust N Z J Surg. – 2003. – V. 73. – 240 p.
20. Warnke, P.H. Growth and transplantation of a custom vascularised bone graft in a man / P.H. Warnke, I.N. Springer, J. Wiltfang [et al] // Lancet. – 2004. – V. 364. – P. 766–770.
21. Yamaguchi, M. Ex vivo expansion of human UC blood primitive hematopoietic progenitors and transplantable stem cells using human primary BM stromal cells

and human AB serum / M. Yamaguchi, F. Hirayama, H. Murahashi [et al] // Cytotherapy. – 2002. – V. 4. – P. 109–118.

22. Triffitt, J.T. Osteogenic stem cells and orthopedic engineering: summary and update / J.T. Triffitt // J Biomed Mater Res. – 2002. – V. 63. – P. 384–389.
23. Martin, G.R. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells / G.R. Martin // Proc Natl Acad Sci U S A. – 1981. – V. 78. – P. 7634–7638.
24. Nakahara, H. In vitro differentiation of bone and hypertrophic cartilage from periosteal-derived cells. Exp Cell Res / H. Nakahara, J.E. Dennis, S.P. Bruder [et al] // – 1991. – V.195. – P. 492–503.
25. Erices, A. Mesenchymal progenitor cells in human umbilical cord blood / A. Erices, P. Conget, J.J. Minguell // Br J Haematol. – 2000. – V. 109. – P. 235–242.
26. Gutierrez-Rodriguez, M. Characterization of the adherent cells developed in Dexter-type long-term cultures from human umbilical cord blood / M. Gutierrez-Rodriguez, E. Reyes-Maldonado, H. Mayani // Stem Cells. – 2000. – V. 18 – P. 46–52.
27. Mareschi, K. Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood / K. Mareschi, E. Biasin, W. Piacibello [et al.] // Haematologica. – 2001. V. 86. – P. 099–1100.
28. Zvaifler, N.J. Mesenchymal precursor cells in the blood of normal individuals / N.J. Zvaifler, L. Marinova-Mutafchieva, G. Adams [et al.] // Arthritis Res. – 2000. – V. 2. – P. 477–488.
29. Jaiswal, N. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro / N. Jaiswal, S.E. Haynesworth, A.I. Caplan [et al.] // J Cell Biochem. – 1997. – V. 64. – P. 295–312.
30. Plate, U. General principle of ordered apatitic crystal formation in enamel and collagen rich hard tissues / U. Plate, S. Arnold, U. Stratmann [et al] // Connect Tissue Res. – 1998. – V. 38 – 149 p.

31. Yamaguchi, A Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. / A. Yamaguchi, T. Komori, T. Suda // Endocr Rev. – 2000. – V. 21. – P. 393–411.
32. Gutierrez Rodriguez M, Reyes Maldonado E, Mayani H (2000) Characterization of the adherent cells developed in Dexter-type long-term cultures from human umbilical cord blood. Stem Cells 18:46–52
33. Mareschi K, Biasin E, Piacibello W, et al (2001) Isolation of human mesenchymal stem cells: bone marrow versus umbilical cord blood. Haematologica 86:1099–1100
34. Zvaifler NJ, Marinova-Mutafchieva L, Adams G, et al (2000) Mesenchymal precursor cells in the blood of normal individuals. Arthritis Res 2:477–488
35. Wobus AM (2001) Potential of embryonic stem cells. Mol Aspects Med 22:149–164
36. Alison MR, Poulsom R, Forbes S, et al (2002) An introduction to stem cells. J Pathol 197:419–423
37. Jaiswal N, Haynesworth SE, Caplan AI, et al (1997) Osteogenicdifferentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 64:295–312 38:149–57
38. Evans CH, Robbins PD (1995) possible orthopaedic applications of gene therapy. J Bone Joint Surg Am 77:1103–1114
39. Oakes DA, Lieberman JR (2000) Osteoinductive applications of regional gene therapy: ex vivo gene transfer. Clin Orthop Relat Res (379 Suppl): 101–112
40. Ashton BA, Allen TD, Howlett CR, et al (1980) Formation of bone and cartilage by marrow stromal cells in diffusion chambers in vivo. Clin Orthop Relat Res (151):294–307
41. Yamaguchi A, Komori T, Suda T (2000) Regulation ofosteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. Endocr Rev 21:393–411
42. Hutmacher DW, Sittinger M (2003) Periosteal cells in bone tissue engineering. Tissue Eng 9:S45–64 Vacanti CA, Kim W, Upton J, et al (1995) The efficacy of

periosteal cells compared to chondrocytes in the tissue engineered repair of bone defects. Tissue Eng 1:301–301–308

43. Friedenstein AJ (1976) Precursor cells of mechanocytes. Int Rev Cytol 47:327–359
44. Nuttall ME, Patton AJ, Olivera DL, et al (1998) Human trabecular bone cells are able to express both osteoblastic and adipocytic phenotype: implications for osteopenic disorders. J Bone Miner Res 13:371–382
45. Triffitt JT, Oreffo ROC (1998) Osteoblast lineage. JAI Press, Inc. Connecticut
46. Dahir GA, Cui Q, Anderson P, et al (2000) Pluripotential mesenchymal cells repopulate bone marrow and retain osteogenic properties. Clin Orthop Relat Res 134–145
47. Bianco P, Riminucci M, Gronthos S, et al (2001) Bone marrow stromal stem cells: nature, biology, and potential applications. Stem Cells 19:180–192
48. Nakahara H, Goldberg VM, Caplan AI (1992) Cultureexpanded periosteal-derived cells exhibit osteochondrogenic potential in porous calcium phosphate ceramics in vivo. Clin Orthop Relat Res (276):291–298
49. Park SR, Oreffo RO, Triffitt JT (1999) Interconversion potential of cloned human marrow adipocytes in vitro. Bone 24:549–554
50. Bahrami S, Stratmann U, Wiesmann HP, et al (2000) Periosteally derived osteoblast-like cells differentiate intochondrocytes in suspension culture in agarose. Anat Rec259:124–130
51. Schantz JT, Hutmacher DW, Chim H, et al (2002) Induction of ectopic bone formation by using human periosteal cells in combination with a novel scaffold technology. Cell Transplant 11:125–138
52. Schantz JT, Hutmacher DW, Ng KW, et al (2002) Evaluation of a tissue-engineered membrane-cell construct for guided bone regeneration. Int J Oral Maxillofac Implants 17:161–174
53. Schwarz MA, Lazo JS, Yalowich JC, Allen WP, Whitmore M, Bergonia HA, et al. Metallothionein protects against the cytotoxic and DNA-damaging effects of

nitric oxide. Proceedings of the National Academy of Sciences. 1995;92(10):4452-6.

54. Makadia HK, Siegel SJ. Poly lactic-co glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011;3(3):1377-97.
55. Ulery, B., Nair, S., and Laurencin, C.T. jets in electrospinning: experiment and modeling *J. Polym. Sci B. Polym. Phys.* 49 (2011) 832.
56. Freiberg, S. and Zhu, Polymer Degradation and Stability X.X. *Int. J. Pharm.* 282 (2004)
57. Ratner, B.D., Hoffman, A.S., Schoen, F.J. and Lemons, J.E.*Biomaterials Science: An Introduction to Materials in Medicine*, 3rd Edition., Academic Press, USA, 2004, p.180.
58. Wiggins, J.S., Hassan, M.K., Mauritz, K.A. and Storey, M.F Polymer Degradation and Stability. *Polymer* 47 (2006) 1960
59. Södergråd, A. and Stolt, M. Preparation of Graphitic Oxide *Prog. Polym. Sci.* 27 (2002) 1123
60. Södergråd, A., Selin, J.F. and Näsman, J.H. Progress in bio-based plastics and plasticizing modifications *Polym. Degrad. Stab.* 51 (1996) 351
61. Li, S.M. and Vert, G.M. *J.* The Real Graphene Oxide Revealed: Stripping the Oxidative Debris from the Graphene-like Sheets. *Mater. Sci. Mater. Med* 1 (1990) 123
62. Rodriguez, E.J., Marcos, B. and Huneault M.A, Manufacturing of PHA as Fibers. In Plastics from Bacteria: Natural Functions and Applications. *J. Appl. Polym. Sci.* 133 (2016) 44152
63. Lyu, S., Schley, J., Loy, B., Lind, D., Hobot, C., Sparer, R. and Untereker, D. Preparation of Graphitic Oxide *Biomacromolecules* 8 (2007) 2301
64. Savioli Lopes M., Jardini A., Maciel Filho R., 2014, Synthesis and characterizations of poly (lactic acid) by ringopening polymerization for biomedical applications, Chemical Engineering Transactions, 38, 331-336, J. Mater. Chem. A, 2013, 1, 13379

65. E.T.H. Vink Lawrence JG, Jones AD, Bhaduri SB et al. / Polymer Degradation and Stability *Polymer* 40 (2003) 403–419
66. T. Maharana et al. Synthesis and characterizations of poly (lactic acid) by ringopening polymerization for biomedical applications,/ Progress in Polymer Science 34 (2009) 99–124
67. Geim, A.K.; Novoselov, K.S. The rise of graphene. Nat. Mater. 2007, 6, 183–191. [CrossRef] [PubMed]
68. Popov, I.A.; Bozhenko, K.V.; Boldyrev, A.I. Is graphene aromatic? Nano Res. 2012, 5, 117–123. [CrossRef]
69. Hummers, W.S.; Offeman, R.E. Preparation of Graphitic Oxide. J. Am. Chem. Soc. 1958, 80, 1339. [CrossRef]
70. Rourke, J.P.; Pandey, P.A.; Moore, J.J.; Bates, M.; Kinloch, I.A.; Young, R.J.; Wilson, N.R. The Real Graphene Oxide Revealed: Stripping the Oxidative Debris from the Graphene-like Sheets. Angew. Chem. Int. Ed. 2011, 50, 3173–3177.
71. Song J, Yang X, Jacobson O, Lin L, Huang P, et al. (2015) Sequential Drug Release and Enhanced Photothermal and Photoacoustic Effect of Hybrid Reduced Graphene Oxide-Loaded Ultrasmall Gold Nanorod Vesicles for Cancer Therapy. ACS Nano 9(9): 9199-9209.
72. Chen L, Zhong X, Yi X, Huang M, Ning P, et al. (2015) Radionuclide I-131 labeled reduced graphene oxide for nuclear imaging guided combined radio- and photothermal therapy of cancer. Biomaterials 66: 21-28.
73. Pourjavadi A, Tehrani ZM, Jokar S (2015) Chitosan based supramolecular polypseudorotaxane as a pH-responsive polymer and their hybridization with mesoporous silica-coated magnetic graphene oxide for triggered anticancer drug delivery. Polymer (Guildf) 76: 52-61.
74. Christopher J. Buchko, Loui C. Chen, Yu Shen and David C. Martin, jets in electrospinning: experiment and modeling *Polymer* 40 (1999) 7397-7407.
75. S. Koombhongse, W. Lin, D.H. Reneker. jets in electrospinning: experiment and modeling *J Polym Sci Part B: Polym Phys* 39: (2001) 2598-2606.

76. Taylor G. Electrically driven jets. *Proceedings of the Royal Society of London A Mathematical and Physical Sciences* 1969;313:453-75.
77. Li F, Zhao Y, Song Y. Core-Shell nanofibers: Nano channel and capsule by coaxial electrospinning. *Nanofibers* 2010:419-38.
78. Theron S, Yarin A, Zussman E, Kroll E. Multiple jets in electrospinning: experiment and modeling. *Polymer* 2005;46:2889-99.
79. Yeo LY, Friend JR. Electrospinning carbon nanotube polymer composite nanofibers. *Journal of experimental nanoscience* 2006;1:177-209.
80. Reneker DH, Chun I. Nanometre diameter fibres of polymer, produced by electrospinning. *Nanotechnology* 1996;7:216.
81. Li D, Xia Y. Electrospinning of nanofibers: reinventing the wheel? *Advanced Materials* 2004;16:1151-70. 82 Beachley V, Wen X. Effect of electrospinning
82. parameters on the nanofiber diameter and length. *Materials Science and Engineering: C* 2009;29:663-8.
83. Touny AH, Lawrence JG, Jones AD, Bhaduri SB. Effect of electrospinning parameters on the characterization of PLA/HNT nanocomposite fibers. *Journal of Materials Research* 2010;25:857-65.
84. Huang L, Nagapudi K, P. Apkarian R, Chaikof EL. Engineered collagen–PEO nanofibers and fabrics. *Journal of Biomaterials Science, Polymer Edition* 2001;12:979-93.
85. Mit-uppatham C, Nithitanakul M, Supaphol P. Ultrafine Electrospun Polyamide-6 Fibers: Effect of Solution Conditions on Morphology and Average Fiber Diameter. *Macromolecular Chemistry and Physics* 2004;205:2327-38.
86. Amiraliyan N, Nouri M, Kish MH. Effects of some electrospinning parameters on morphology of natural silk-based nanofibers. *Journal of Applied Polymer Science* 2009;113:226-34. 87 Carrizales C, Pelfrey S, Rincon R, Eubanks TM, Kuang A, McClure MJ, et al. Thermal and mechanical properties of electrospun PMMA, PVC, Nylon 6, and Nylon 6, 6. *Polymers for Advanced Technologies* 2008;19:124-3

87. Zong X, Kim K, Fang D, Ran S, Hsiao BS, Chu B. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *Polymer* 2002;43:4403-12.
88. Jacobs V, Anandjiwala RD, Maaza M. The influence of electrospinning parameters on the structural morphology and diameter of electrospun nanofibers. *Journal of Applied Polymer Science* 2010;115:3130-6.
89. Lavielle N, Popa A-M, de Geus M, Hébraud A, Schlatter G, Thöny-Meyer L, et al. Controlled formation of poly (ε-caprolactone) ultrathin electrospun nanofibers in a hydrolytic degradation-assisted process. *European Polymer Journal* 2013;49:1331-6.
90. Gupta P, Elkins C, Long TE, Wilkes GL. Electrospinning of linear homopolymers of poly (methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent. *Polymer* 2005;46:4799-810.
91. McKee MG, Wilkes GL, Colby RH, Long TE. Correlations of solution rheology with electrospun fiber formation of linear and branched polyesters. *Macromolecules* 2004;37:1760-7
92. Deitzel JM, Kleinemeyer J, Harris D, Beck Tan NC. The effect of processing variables on the morphology of electrospun nanofiber and textiles. *Polymer* 2001;42:261-72.
93. Ramakrishna S, Fujihara K, Teo W-E, Lim T-C, Ma Z. An introduction to electrospinning and nanofibers: World Scientific; 2005..
94. Subbiah T, Bhat G, Tock R, Parameswaran S, Ramkumar S. Electrospinning of nanofibers. *Journal of Applied Polymer Science* 2005;96:557-69.
95. Pham QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Engineering* 2006;12:1197-211.
96. Buchko CJ, Chen LC, Shen Y, Martin DC. Processing and microstructural characterization of porous biocompatible protein polymer thin films. *Polymer* 1999;40:7397-407.

97. Frenot, A., Chronakis, I.S., Polymer Nanofibers Assembled by Electrospinning. Current Ppinion in Colloid & Interface Science, 2003. 8(1): p. 64-75.
98. Liu, Y., Dong, L., Fan, J., Wang, R., Yu, J.-Y., Effect of Applied Voltage on Diameter and Morphology of Ultrafine Fibers in Bubble Electrospinning. Journal of Applied Polymer Science, 2011. 120(1): p. 592-598.
99. Niu, H., Lin, T., Fiber Generators in Needleless Electrospinning. Journal of Nanomaterials, 2012. 2012: p. 12
100. .Luo, C.J., Nangrejo, M., Edirisinghe, M., A Novel Method of Selecting Solvents for Polymer Electrospinning. Polymer, 2010. 51(7): p. 1654-1662
101. De Vrieze, S., Van Camp, T., Nelvig, A., Hagström, B., Westbroek, P., De Clerck, K., The Effect of Temperature and Humidity on Electrospinning. Journal of Materials Science, 2009. 44(5): p. 1357.
102. Pakravan, M., Heuzey, M.-C., Ajji, A., A Fundamental Study of Chitosan/PEO Electrospinning. Polymer, 2011. 52(21): p. 4813-4824
103. Van der Schueren, L., De Schoenmaker, B., Kalaoglu, Ö.I., De Clerck, K., An Alternative Solvent System for the Steady State Electrospinning of Polycaprolactone. European Polymer Journal, 2011. 47(6): p. 1256-1263.