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Tesi di Laurea Magistrale

Optimization and characterization of electrospun composite fibers containing bioactive glass particles



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Abstract

The aim of this master's thesis project is to optimize and characterize composite electrospun fibers containing bioactive glass particles.

Electrospinning is a common and versatile technique used to create nanofibrous mats for various applications including the biomedical field, whereas bioactive glasses (BGs) are ceramic materials that have the ability bond to living tissue, principally bone, through the creation of a hydroxyapatite layer, moreover they could contain different ions such as boron or copper, that have been chosen for this work, to improve angiogenetic, anti-inflammatory or antibacterial properties.

The fist part of this thesis, performed at Politecnico di Torino (Turin, Italy), consisted in optimizing the synthesis process, via sol-gel method, of BG nanoparticles, containing copper and boron, to avoid aggregation and permit a good ions incorporation. The particles were characterized using SEM, EDS, XRD, FTIR, BET and bioactivity analysis, both before and after the immersion in an acetic acid solution to verify the preservation of ions incorporation and bioactivity of the particles in the acid later used for the electrospinning solution.

The second and final part of the thesis was performed in Friedrich-Alexander-Universität Erlangen-Nürnberg (Erlangen, Germany), and consisted in the preparation of composite poly(ϵ -caprolactone)/BG fibers using acetic acid as solvent, and their characterization by SEM, EDS, FTIR, water contact angle measurements, mechanical tensile test and bioactivity analysis.

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1 Introduction

1.1 Bioactive glasses

The bioactive glasses are a group of reactive ceramic materials, the first that have been developed are principally composed by SiO_2 , CaO, Na_2O and P_2 O_5 , with an high content of silica, studied for their biocompatibility and bioactivity properties.

A bioactive material is somehow a midway between an inert material and a resorbable material, its principal features are linked to the ability of the material to bond to living tissue and, in general, generate a biological response by the living organism, bioactive glasses, for example, directly bond to bone tissue thanks to a biologically active layer of hydroxyapatite (HA) that is chemically equivalent to the mineral phase of the bone [1].

A bioactive glass, to function as a suitable biomaterial, must respect a list of desired parameters:

- must be non-toxic and promote cell proliferation and adhesion.
- its crystalline phases must not induce cytotoxicity or block any bioactive process inside the tissue.
- must form an HA layer when put in contact with biologic fluids or simulated body fluid (SBF) solutions.
- must exhibit mechanical properties that are comparable to those of the tissue that it has to replace.
- could possess interconnected and adequate porosity to support vascular growth.
- should be cost effective for commercialization [2].

The first ever attempt to create a ceramic material that could be used to help the bone tissue regeneration was made by L.L. Hench around 1970, thanks the founding of the US army medical R and D command.

The hypothesis beyond this project was that the materials usually used for body implants, synthetic polymers and metals, are not the perfect material for the human body because, the firsts have a low mechanical strength that cannot withstand the stresses required for many applications, while the seconds, despite their high resistance and mechanical strength, they have a high corrosion rate and low biocompatibility, and the diffusion of metal ions can lead to allergic reaction, rejections and scar formation in biological tissue after the implant, so it was necessary to create a material that was able to form a HA layer in vivo due to the fact that bone contains a HA component and such material may not be rejected by the body.

The composition of this material is 45% SiO₂ - 24.5% Na₂O - 24.5% CaO - 6% P_2O_5 and by the end of 1971 it was proven to form an interfacial bonding with the bone tissue, due to the formation of a HA layer. This material is called 45S5 Bioglass[®] and it's still used today as a starting point for several studies as it joins readily even to soft tissues [3].

Since then, a lot of modification of composition and addition of different ions were made trying to find the "perfect" bioactive glass. An examples could be the glass-ceramic materials that belong to the A/W class, such as the Cerabone[®] by T. Kokubo (glass composition 34% SiO₂, 44.7% CaO, 4.6% MgO, 16.2% P₂O₂, 0.5% CaF₂) with the common characteristic of nucleating around apatite and wollastonite and having better mechanical proprieties. [4] Another example could be the one proposed by Beall, not having an amorphous phase of alumina, or Biovert[®] with the nucleation of mica and apatite and with some composition in which silica was completely replaced by P₂O₅ [5] [6].

1.2 Bioactivity and HA formation process in bioactive glasses

The bioactivity process in 45S5 like bioactive glasses could be resumed in five simple steps.

- 1. Rapid diffusion-controlled exchange of ions between the glass network modifiers and the body fluid, Ca^{2+} and Na^{+} ions pass from the glass to the solution and H_3O^{+} ions enter the glass.
- Partial dissolution of silica molecules, Si(OH)₄ at the surface, resulting in the breaking of Si-O-Si bonds and formation of silanol groups Si-OH thanks to the local pH increase due to the previous step (Si-O-Si + H₂O = Si-OH + OH-Si).

- 3. Condensation of silica layer and formation of a hydrated amorphous silica gel layer (SiO₂) due to polymerization process.
- Incorporation of calcium (Ca²⁺) and phosphate (PO₄³⁻) groups by the SiO₂ layer forming an amorphous calcium phosphate layer at the interface between the glass and the solution.
- Crystallization of the amorphous film into HA thanks to the migration of OH⁻ and other carbonate ions from the solution to the glass [2] [7] [8].

After the HA formation adsorption of grow factors, adhesion protein, attachment, proliferation and differentiation of osteoprogenitor cells are the biological responses that lead to the attachment of the glass to the bone.

1.3 Bioactive glass synthesis

Conventionally the glasses can be prepared using two different techniques, the first one is the melting technique, in which the glass is obtained by melting a mixture of materials with a subsequent solidification; the second one is the sol-gel technique in which organic liquid precursors are used for the gelation and subsequent drying and calcination to obtain the glass.

1.3.1 Melting technique

It's the most common way to obtain a glass, it consists in a fusion step of different oxides followed by their quenching. The quenching is a rapid cooling of a material in water, oil, air or other fluids like liquid nitrogen or mercury, to obtain certain properties, that, in this case, are the avoiding of any crystallization.

Before the fusion step, the initial ingredients are firstly milled to obtain homogeneous particle size and to break aggregates; usually the milling is done in a wet medium, like acetone, or just water if the raw materials are not highly hygroscopic, and then the obtained pounder mixture is dried in air.

The mixture is then melted in a furnace, the temperatures can go up to 1500°C and the melting temperature must be maintained for 2 h so that the gaseous substances are released and a homogeneous molten material is obtained.

The next steps are to cast the melted material into a mould, quench in the appropriate liquid or gas, and in the end anneal at 500-700°C to remove the internal stresses [2] [9].

1.3.2 Sol-gel technique

The sol-gel technique consists in the dispersion of colloidal particles in a liquid, the hydrolysis and polymeric condensation generally of an organometallic precursor, lead to the formation of a 3D network that later forms a gel.

To form a bioactive glass using this technique, six simple passage are required:

- mixing all the precursors together (they can be alkoxides or organometallics).
- hydrolysis of liquid precursors with de-ionized water, this allows the rise of silanol groups (Si(OH)₄) that interact with each other forming a silica network via polycondensation.
- after the formation of the gel, the gelatinization process follows, during this process an instantaneous increase of viscosity is observed.
- aging process by hydrothermal treatment (60-80°C/48h), decrease of porosity and increase of strength is observed.
- drying process (150-180°C/3-6h)
- calcinating process (600-700°C/2-5h) to stabilize the glass [9].

1.4 Synthesis of silica nanoparticles by sol-gel methods

For the purposes of this master thesis it is useful to search for a method to create bioglass nanoparticles (BGN), instead of porous scaffolds, via sol-gel. Among others, two main ways are possible: the base-catalysed method (usually called Stöber method), and the Acid/base co-catalysed method [10].

1.4.1 Stöber method

Since silicon alkoxides tend to form 3D structures under acid condition and individual particles under basic conditions, a simple way to create monodispersed silica nanoparticles is to produce a base-catalysed sol-gel routine.

The Stöber method consists in the creation of a solution composed by cetyltrimethylammonium chloride (CTAC), deionized water, methanol and 28% aqueous ammonia solution, and, after a short mixing time, in the addition of tetraethoxysilane (TEOS). The ammonia solution serves as a catalysator for the entire reaction, helping the formation of a turbid solution after ca. 10 minutes, indicating particles formation.

The mixture is then aged at room temperature for 20h, centrifuged to separate the products, washed with methanol and then dried at 60°C for a day. At the end, to remove the surfactant, is necessary a calcination process in air at 550°C for 10h [11].

This method could also be used to form BGN, apart from TEOS, also metal ions precursors are added to the solution, and it's kept under continuous agitation. The process of the introduction of metal ions could impact the surface charge of SiO₂ nanoparticles that could lead to aggregation, inhomogeneity in size and irregular shapes of the formed BGN. To avoid these problems, it's common practice to modify the Stöber method, the most common modification are listed below:

- there is a threshold amount of Ca that can be incorporated into the BGN, a plateau is reached when Ca/Si atomic ratio is 2
- time of addition of calcium precursor: it can be seen that that the time of addition of calcium precursor influences the aggregation of particles and incorporation of Ca ions inside the BGNs: a short

addition time leads to more aggregated particles and higher concentration of Ca ions, while long addition time leads to monodispersed particles but with a low presence of Ca ions;

- the variation of NH4OH concentration impacts the size of the particles, a higher concentration of ammonia promotes the creation of bigger, monodispersed nanoparticles;
- organic species can be used as steric barriers to improve the dispersity;
- ultrasounds (US) can be used to help the formation of monodispersed particles;
- formation of the nanoparticles from two different solutions, the first one containing just ethanol and TEOS, and the other one containing water, ethanol and NH₄OH [10] [12] [13] [14] [15] [16] [17].

1.4.2 Acid/base co-catalyzed method

Another synthesis method for BGNs formation can be done in presence of an acid catalyst, however, a basic catalyst is still needed to induce gel formation.

In this synthesis, TEOS and metal ion precursors are mixed under acid conditions and then, the pH is raised to accelerate the formation of nanoparticles.

In contrary of the previous method, here, small colloidal particles tend to form a 3D gelled structure due to the acid conditions, this could result in polydispersed and more agglomerated morphology.

To improve the monodispersity of BGN a weak organic acid or non-ionic surfactant such as poly(ethylene glycol) (PEG) or Pluronic can be used to act as a steric barrier [10].

1.5 Effect of different ions in BG properties

The most common network former in BGs are silica (SiO₂), phosphorus pentoxide (P_2O_5) and boron trioxide (B_2O_3). In biomedical applications silica based bioactive glasses containing sodium, calcium and phosphorus in different proportions are widely used, but it's also common to observe BGs composition containing other additional elements such as zinc, magnesium, boron, copper, silver, cobalt and many others.

It is already estabilished that calcium and phosphorus play an important role in the bioactivity and bone formation process since they are the main components in HA, but for the purpose of this master thesis these are not the only incorporated ions. Due to their specific characteristic copper and boron ions have also been added, following the atomic composition of reference [18].

Copper (Cu) is a trace element, and this means that, if used in the right amount, could be very beneficial to the body and bone formation process. It has been proven that BGs containing Cu²⁺ ions help wound healing and angiogenesis thanks to copper-sensitive pathways, the same utilized by hypoxia, that regulate factors that are essential in these process, such as vascular endothelial growth factor (VEGF) or fibroblast growth factor-2 (FGF-2).

Helping the tissue regeneration and angiogenesis is not the only positive effect of Cu^{2+} ions, a lot of studies focus on their strong antibacterial properties, principally against *Escherichia coli* and *Staphylococcus aureus*, showing that, the increase in Cu^{2+} in the particles, and successively in the surrounding medium, leads to the penetration and killing of bacterial cells. It is still necessary to pay attention though, because concentration of Cu^{2+} ions higher than a certain limit, could lead to cytotoxicity and unfavourable condition for cell attachment [19] [20] [21] [22].

Boron (B) is another trace element for in the human body it has been proven that plays an important role in stimulating the bone and wound healing in vivo and angiogenesis in vitro, it helps the release of growth factors and cytokine such as IL-6 and bFGF for pro-angiogenic purposes, and also increase the extracellular matrix turnover [23] [24].

It is important to notice that, being boron a trace element, it must be present in the body in physiological amount, Brown et al. [25] demonstrated as an increasing in B_2O_3 amount greater then 1/3 of SiO₂ in a classic 45S5S glass could inhibit cell proliferation especially in static culture conditions.

1.6 Electrospinning

The electrospinning technique is one of the methods for the fabrication of nanofibrous mats. This technique is lately preferred to solvent casting and phase separation methods because the nanofibers produced possess a high surface to area volume ration and a larger number of inter-/intra fibrous pores, plus it is a simple and cost effecting technique. [26] It can be applied in almost every field, from biomedical [27], to environmental [28], to the production of biological nanosensors [29], the ability to create nanostructures from a huge amount of polymers is, in fact, increasing the numbers of scientists from around the globe, interested in this technology.

It is important to say that the first time the electrospinning technique appeared in literature was more than a century ago with the application of Coley [30], but it started to become of great interests just in the 1990s with the firsts commercial application and after 2000 with a strong increase in scientific publications [31].

1.6.1 Mechanism

The basic electrospinning setup comprises four different parts:

- a syringe containing the polymer solution
- a metallic needle
- a power supply to adjust voltage
- a metallic collector

The basic principle behind this technology is the application of a tension between the needle and the collector, this tension causes instability within the polymer solution inside the syringe as a result of induced charges. When the applied electric field is able to overcome the surface tension forces of the solution a jet will originate from the Taylor cone causing the formation of polymer fibers, that will be collected by the collector at the other end of the instrument after travelling for few seconds in two different zones. The first zone is called stable zone, because the jet travel in a direct route, in the second zone, instead, the jet become thinner and unstable. It is important to say that the polymer flow is kept constant for all the process thanks to a pump linked to the previously mentioned syringe.

1.6.2 Parameters

There are different parameters that could influence the final result on an electrospinning process, simplifying they could be divided in electrospinning parameters (such as applied electric field, distance needle-collector, flowrate, needle diameter), solution parameters (such as used solvent, polymer concentration, viscosity and solution conductivity), and environmental parameters (such as humidity and temperature) [26]. A list of parameters and the consequence of their changes will be now presented.

Voltage: the applied voltage is the direct parameter that consents the formation of a jet expulsion from the Taylor cone, so it is natural that the minimum voltage to form a jet is directly linked to the used polymer and therefore, the surface tension of the solution. It has been proven that a higher voltage could lead to the formation of smaller diameter nanofibers linked to higher repulsive forces within the polymer jet [32], by the way, an higher voltage could also lead to the creation of beads or beaded nanofibers due to the decrease in the size of the Taylor cone and an increase of jet velocity, if the same flowrate is maintained.

It is important to say that some researchers are in discord with this results because of reported bigger diameter fibers with an increased voltage, due to a greater quantity of polymer ejected [33].

 Flowrate: the flowrate influences the morphology of the electrospun fibers, beadless uniform fibers could be prepared reaching a critical flowrate value, value that variate with the polymer system. A minimum flowrate is necessary to maintain a balance between the leaving polymer solution and the replaced one but if the flowrate is increased over a certain value this could lead to the creation of bigger diameter fibers, surface defects such as beads and ribbon-like structures, unspun droplets, and an increase on pore size and diameter [34].

- Working distance: de distance between the needle and the collector influences the solvent evaporation. A critical distance, in which the solvent has enough time to evaporate, is needed to prepare smooth and uniform nanofibers. Shorter distances could originate thick and defective fibers whereas longer distances could lead to thinner fibers and discontinuity [35].
- Polymer concentration and solution viscosity: the uniaxial stretching
 of the charged jet is directly linked to polymer concentration, and,
 therefore, viscosity of the solution. With low concentration values,
 and high or low viscosity values, the applied tension could cause the
 breaking of the polymer chains leading to the formation of beaded
 fibers, furthermore increasing concentration beyond a critical value,
 could lead to hamper the flow of the solution throughout the needle
 due to the drying of the polymer solution inside the needle itself [36].
- Solution conductivity: in general, an increase in conductivity leads to thinner fibers formation due to the increase in the charge of the initial droplet. In solutions with low conductivity the surface of the droplet will have no charge to form a Taylor cone leading to the nonformation of the mat, whereas if the conductivity increase to much the fibers will show an irregular diameter due to the high instability of the polymeric jet [37].
- Solvent: selecting the right solvent is a really important passage for the formation of smooth and beadless nanofibers, the parameters to keep in mind are the ability of the solvent to dissolve the chosen polymer, not all the solvents are capable to completely dissolve all

polymers; the surface tension and, at last, the volatility. A high volatility could lead to the drying of the solution inside the needle or at the needle tip blocking the electrospinning process [32] [38]. In the last years began to be more and more popular the idea of "Green electrospinning" and the use of non-toxic/benign solvents, solvents that, have a lot of vantages from the environmental, safety for the lab workers and presence of residuals of toxic solvents in the electrospun mat. Those solvents, some examples are acetic acid or formic acid, could also help to blend synthetic polymers with natural ones without the risks of denaturation that usually occur when using harsh solvents [47].

- Temperature: the temperature mostly affect the evaporation rate of the solvent, it has been proven that increasing temperature to the boiling point of the solvent could cause pores inside the fibers thanks to the evaporation of the molecules present on the fiber surfaces.
- Humidity: humidity could cause changes in the nanofibers diameters by controlling the solidification process of the jet, higher values of humidity lead to a decrease of the fibers diameter [39].

1.7 Addition of glass nanoparticles

The main problem in the addition of glass particles inside the electrospun fibers is the difficulty in reaching a good and uniform dispersion of the particles, usually a formation of agglomerates could occur due to the tendency of the particles to combine into aggregates in order to diminish the surface area [40]. Bigger aggregates tend to form defects in the mat, leading to an easier formation of crack and consequently diminish the mechanical properties of the composite material, if compared to the neat polymer. [41]. It is important to notice that smaller particles tend to aggregate more even before the addition inside the polymeric solution, and this could lead to a more difficult dispersion. [42]. Optimization of the bioactive glass nanoparticles synthesis, mortar passages and addition methods could help a better dispersion of the nanoparticles inside the nanofibers.

2 Materials and Methods

2.1 Glass composition

Before starting the optimization of the BGs synthesis, it was necessary to choose an ideal composition. For this purpose we thought that the reagents and proportion of the SBCu glasses of reference [18] could be perfect to have an already studied and bioactive composition in order to shift the focus of the work more on the synthesis process, avoid or reduce aggregation and improve the spherical geometry of the particles other than on the glass composition.

The main components of the glass were SiO₂, P₂O₅, CaO, CuO and B₂O₃, in order to create nanoparticles starting from a classic SiO₂-CaO-P₂O₅ composition (77S glass), widely studied in literature, with the addition of copper and boron to increase and confer angiogenetic and antibacterial properties, this is an innovative glass composition, that has been developed by Enrica Vernè's research group (DISTAT-Politecnico di Torino).

The percentage (in weight) of each component, is now listed:

- 62 wt% SiO₂: using tetraorthosilicate (TEOS) C₈H₂₀O₄Si at 99% (Sigma Aldrich) as reagent
- 9 wt% P_2O_5 : using triethyl phosphate (TEP) $C_6H_{15}O_4P$ at 99% (Alfa Aesar) as reagent
- 15 wt% CaO: using calcium nitrate tetrahydrate Ca(NO_3)_2 \cdot 4 H_2O as reagent
- 5 wt% CuO: using copper nitrate trihydrate Cu(NO_3)_2 \cdot 3 H_2O (Fluka) as reagent
- 15 wt% $\mathsf{B}_2\mathsf{O}_3$: using boric acid $\mathsf{H}_3\mathsf{BO}_3$ at 99% (Sigma Aldrich) as reagent

Due to the need of having a comparison with a more commonly synthetized glass (SiO_2 -CaO- P_2O_5), a control glass is also produced, the percentage of each component is now listed:

- 77 wt% SiO₂: using tetraorthosilicate (TEOS) C₈H₂₀O₄Si at 99% (Sigma Aldrich) as reagent
- 9 wt% P₂O₅: using triethyl phosphate (TEP) C₆H₁₅O₄P at 99% (Alfa Aesar) as reagent
- 14 wt% CaO: using calcium nitrate tetrahydrate Ca(NO_3)_2 \cdot 4 H_2O as reagent

2.2 Bioactive glass synthesis

The main aim of the first part of this master's thesis was to improve the shape and aggregation state of the nanoparticles, to reach this result, after a period of bibliographic research useful to find out what was the state of the art in BG nanoparticles synthesis process, as already discussed in the introduction chapter of this thesis; different synthesis processes have been tried, for the first four synthesis no copper or boron have been used, to reduce the level of complexity of the system and focus more on which method was the most promising.

2.2.1 S1_ab (S1)

The first synthesis has been made following the process of [18] (synthesis 1) to have a fresh control.

- 30 ml ethanol (EtOH)
- 7.2 ml H₂O
- 1.2 ml HNO₃ (2M)
- 11.2 ml TEOS \rightarrow mix 1h
- Dropwise NH₄OH (2M) addition until pH 8/9 is reached

- 0.84 ml TEP→ mix 30'
- 2.31 g Ca \rightarrow mix 30'
- 48 h in heater 60°C
- Oven 2 h at 700°C slow temperature heating rate (5 °C/min)

2.2.2 S1_ab_Ca3h (S2)

Previous studies have demonstrated that a longer time before the addition of the calcium precursor, can lead to the formation of less aggregated nanoparticles [12] [43], with the negative effect of reducing the quantity of ions present inside the nanoparticles themselves. A good compromise is proven to be the adding of the calcium salt after 3 hours. So, the second synthesis attempt is a slight modification of the previous process, adding the calcium after 3 hours of magnetic stirring, instead of 30 minutes.

The synthesis process is described as follow:

- 30 ml EtOH
- 7.2 ml H₂O
- 1.2 ml HNO₃ (2M)
- 11.2 ml TEOS \rightarrow mix 1h
- Dropwise NH₄OH (2M) addition until pH 8/9 is reached
- 0.84 ml TEP \rightarrow mix 3h
- 2.31 g Ca \rightarrow mix 30'
- 48 h in heater 60 °C
- Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.3 S2_b (S3)

In literature, silica nanoparticles are synthetized very often mixing two different solutions, the first one containing just EtOH and TEOS, and the other one containing water, EtOH and NH₄OH. Apart from the use of two different solutions, the nanoparticles have been washed in distilled water and in EtOHto remove the non-incorporated ions and avoid charge mediated aggregation. Another difference from the previous synthesis process is the total absence of nitric acid, due to the fact that some studies showed that the use of nitric acid, so the presence of a lower pH value during the first part of synthesis process, could lead to particle aggregation [12] [17] [44].

- 1st solution:
 - 93 ml EtOH
 - 11.2 ml TEOS→mix 30'
- 2nd solution:
 - 46 ml H₂O
 - 30 ml EtOH
 - 17 ml NH₄OH 33%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- 0.84 ml TEP→ mix 30'
- 2.31 g Ca→ mix 1 h 30'
- Centrifuge 5min at 7000rpm
- Wash with distilled water
- Centrifuge 5 min at 7000rmp
- Wash with ethanol
- Centrifuge 5 min at 7000rpm
- 48h in heater 60 °C
- Oven 2 h at 700 °C slow temperature heating rate (5°C/min)



Figure 2.2.3.1: Synthesis S1, S2 and S3 inside drying inside the stove

2.2.4 S2_b_Ca3h (S3.1)

This synthesis is a mix of the previous two (S1_ab_Ca3h and S2_b), two solutions are used, and the calcium salt is added after 3h. The final solution is not washed due to the low presence of ions found in the previous synthesis.

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
 - 8.5 ml NH₄OH 28%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- 0.42 ml TEP \rightarrow mix 3h

- 1.16g Ca→mix 1 h 30'
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- oven 2 h at 700 °C slow temperature heating rate (5°C/min)

2.2.5 S2_b_Ca24h

This synthesis is like the one just described S2_b_Ca3h (S3.1), but after the addition of each element precursor, the solution was kept on magnetic stirring for 24 h instead of 1 h 30', to see if there were substantial differences in ions incorporation.

2.2.6 S2BCu_b (SBCu)

This synthesis is identical to S2_b_Ca3h (S3.1) but in order to increase the complexity of the system and verify if also boron and copper doped glass could be synthetized using this synthesis, boron and cupper precursor were also added. No problems raised during the synthesis of BG containing both boron and copper so, from now, on boron and copper were added for every synthesis.

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
 - 8.5 ml NH₄OH 28%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'

- 0.5 2ml TEP \rightarrow mix 3 h
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'
- 0.65 g B→mix 30'
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)



Figure 2.2.6.1: Samples S3.1 and SBCu inside the oven for the annealing process

2.2.7 S2BCu_b24h

As previously written for the synthesis S2_b_Ca24h, to see if there were a substantial change in ions incorporation, the synthesis S2BCu_b was kept under magnetic stirring for 24 h, after the addition of all the precursors.

2.2.8 S2BCu_b_no_sol (S4)

Since, after the centrifugation, a high percentage of ions can be noticed in the solution, thanks to the blue colour of copper ions, the solution is centrifuged just after the formation of the silica nanoparticles and almost all the liquid part is removed, prior to the adding of the other elements. No centrifugation is done at the end of the process.

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
 - 8.5 ml NH₄OH 28%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- Centrifuge 5 min at 7000rpm
- Remove the liquid part of the solution
- 0.52 ml TEP→mix 30'
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'
- 0.65 g B \rightarrow mix 30'
- 48 h in heater 60°C
- oven 2 h at 700°C slow temperature heating rate (5 °C/min)

2.2.9 S2BCu_b_no_sol_cent (S4 cent)

This synthesis is similar to S2BCu_b_no_sol (S4), but in this case a centrifugation step is added at the end (as indicated by "cent" in the synthesis name). The centrifugation step could be useful to remove as much liquid solution as possible before the drying process, this is necessary because in previous synthesis could be seen as more liquid could lead to more aggregated prarticles.

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
 - 8.5 ml NH₄OH 28%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- Centrifuge 5 min at 7000rpm
- Remove the liquid part of the solution
- 0.52 ml TEP \rightarrow mix 3h
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'
- 0.65 g B→mix 30'
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- oven 2 h at 700 °C slow temperature heating rate (5 °C/min)



Figure 2.2.9.1: Synthesis S4 before and after the adding of copper

2.2.10 S2BCu_b_end (S5)

In order to gelify the sol after the addition of all precursors can be used another strategy, for this synthesis as suggested by some works found in the literature and following a strategy also followed in the second synthesis of [18], NH₄OH was added after all the precursors [42].

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:

- 23 ml H₂O
- 15 ml EtOH
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- 0.52 ml TEP \rightarrow mix 3 h
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'
- 0.65 g B→mix 30'
- 8.5 ml NH₄OH 28% → mix 30'
- 48 h in heater 60 °C
- oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.11 S2BCu_b_end_cent (S5 cent)

The same as S2BCu_b_end (S5) but centrifuged at the end, analogously to S2BCu_b_no_sol_cent.

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- 0.52 ml TEP \rightarrow mix 3 h
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'
- 0.65 g B→mix 30'
- 8.5 ml NH₄OH 28% → mix 30'
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- oven 2 h at 700°C slow temperature heating rate (5 °C/min)

2.2.12 S2BCu_b_end_stufa (S5 stufa)

After 48 h, the glasses produced by S2BCu_b_end synthesis were not completely dried, so that synthesis was modified increasing the drying time in heater (3 days).

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- 0.52 ml TEP \rightarrow mix 3 h
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'
- 0.65 g B→mix 30'
- 8.5 ml NH₄OH 28% → mix 30'

- 72 h in heater 60 °C
- Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.13 S1BCu_b (S6)

Following some literature results and data already collected in the previous synthesis, in this synthesis there was a return to a one solution method, used for S1_ab (S1), but without the nitric acid, pH is now raised from 8 to 12, because a higher pH seems to increase nanoparticles diameter, and an higher diameter helps the non-aggregation of the nanoparticles. [12]

The synthesis process is described as follow:

- 30 ml EtOH
- 7.2 ml H₂O
- 11.2 ml TEOS \rightarrow mix 1 h
- Dropwise addition of NH₄OH (2M) until a pH 12 is reached
- 1.04 ml TEP→mix 30'
- 2.31 g Ca \rightarrow mix 30'
- 0.74 g Cu→mix 30'
- 1.3 g B→mix 30'
- 48 h in heater 60 °C
- oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.14 S1BCu_b_cent (S6 cent)

The same process used for S1BCu_b (S6) but centrifuging at the end.

- 30 ml EtOH
- 7.2 ml H₂O
- 11.2 ml TEOS \rightarrow mix 1 h
- Dropwise addition of NH₄OH (2M) until a pH 12 is reached
- 1.04 ml TEP→mix 30'
- 2.31 g Ca → mix 30'
- 0,74g Cu→mix 30'
- 1.3 g B→mix 30'
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.15 S1BCu_b_US (S7)

Since ultrasound (US) are widely used in the synthesis of non-aggregated silica nanoparticles, a change in S1BCu_b (S6) process is been made to see the effect of US while mixing, note that it was impossible to use magnetic stirrer so the mixing was made mechanically using the setup showed in Fig. xxx. [13] [14] [15] [16]

- 30 ml EtOH
- 7.2 ml H₂O
- 11.2 ml TEOS \rightarrow mix 1 h
- Dropwise addition of NH₄OH (2M) until a pH 12 is reached
- 1.04 ml TEP→ mix 30'
- 2.31 g Ca \rightarrow mix 30' in US bath
- 0.74 g Cu \rightarrow mix 30' in US bath

- 1.3 g B \rightarrow mix 30' in US bath
- 48 h in heater 60°C
- Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)



Figure 2.2.15.1: Synthesis S7 while mixed in sonic bath

2.2.16 S1BCu_b_US_cent (S7 cent)

Same process of S1BCu_b_US (S7) but centrifuging at the end.

- 30 ml EtOH
- 7.2 ml H₂O
- 11.2ml TEOS \rightarrow mix 1 h
- Dropwise addition of NH₄OH (2M) until a pH 12 is reached
- 1.04 ml TEP→ mix 30'
- 2.31 g Ca \rightarrow mix 30' in US
- 0.74 g Cu→mix 30' in US

- 1.3 g B \rightarrow mix 30' in US
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.17 S3BCu_b (S8)

This synthesis is a variation of S2Bcu_b_no_sol (S4) synthesis, in details a drying step in heater (for 48 h at 60 °C) was introduced after the first centrifugation. This variation of the previous synthesis permit the formation of pure silica nanoparticles, avoiding, in theory aggregation, which can be triggered by the charge of the dopant ions, in the first step.

- 1st solution:
 - 46.5 ml EtOH
 - 5.6 ml TEOS→mix 30'
- 2nd solution:
 - 23 ml H₂O
 - 15 ml EtOH
 - 8.5 ml NH₄OH 28%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- Add 15 ml H2O
- 0.52ml TEP→mix 3 h
- 0.925 g Ca→mix 30'
- 0.37 g Cu→mix 30'

- 0.65 g B→mix 30'
- 48 h in heater 60 °C
- oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.18 S3BCu_b24h

As previously done for other synthesis, this had the exact same process of S3BCu_b, but the solution was kept under magnetic stirring for 24 h after the addition of all precursors, to see if there were substantial differences in ions incorporation.

2.2.19 S1BCu_ab_US (S9)

This synthesis is the same as synthesis S1_ab (S1) but reaching a higher pH, due to the already discussed effect of a creation of bigger nanoparticles and successive formation of fewer clusters, and using US bath while mixing to see the effects of these parameters on the acid/base co-catalyzed synthesis of [18].

- 30 ml EtOH
- 7.2 ml H₂O
- 1.2 ml HNO₃ (2M)
- 11.2 ml TEOS \rightarrow mix 1 h
- Dropwise addition of NH₄OH (2M) until a pH 12 is reached
- 1.04 ml TEP→ mix 30'
- 2.31 g Ca \rightarrow mix 30' in US bath
- 0.74 g Cu \rightarrow mix 30' in US bath
- 1.3 g B \rightarrow mix 30' in US bath
- 48 h in heater 60 °C

• Oven 2 h at 700 °C slow temperature heating rate (5 °C/min)

2.2.20 Final synthesis

Between all this different synthesis process, the best is proven to be, as explained in the chapter "Results", S2BCu_b_no_sol_cent (S4 cent), called now for convenience SBCu4.

Having selected the best synthesis method, two further optimization steps were tried, SBCu4_freezer and SBCu4_lyo, the only difference between these two synthesis methods and the selected SBCu4 synthesis is in the drying step. Some literature results has shown that it is possible to avoid the aggregation of the glass particles by freezing and lyophilizing the gel, the first one was left for 2 days in freezer at -18°C while the latter was put in a lyophilizer, after the drying, the annealing and calcination process in the oven was the same.

In order to evaluate the effect of boron and copper ions presence, a glass without B and Cu ions was synthetized using the same synthesis as SBCu4 and used as control.

- 1st solution:
 - 93 ml EtOH
 - 11.2 ml TEOS→mix 30'
- 2nd solution:
 - 46 ml H₂O
 - 30 ml EtOH
 - 17 ml NH₄OH 28%
- Pour over the 2^{nd} solution into the 1^{st} one \rightarrow mix 30'
- Centrifuge 5 min at 7000rpm
- Remove the liquid part of the solution
- 0.84 ml TEP \rightarrow mix 3 h
- 2.31 g Ca→mix 1 h 30'

- Centrifuge 5 min at 7000rpm
- 48 h in heater 60 °C
- oven 2 h at 700°C slow temperature heating rate (5 °C/min)



Figure 2.2.20.1: Synthesis S4 and SBCu4 at the end of the centrifugation process

2.3 Bioactive glass nanoparticles characterization

2.3.1 Morphological characterization

The morphological characterization of the BG particles was made using the Field Emission Scanning Electron Microscopy (FE-SEM) technique. The used microscope was a FE-SEM Gemini SUPRATM 40 (Zeiss, Germany). The samples were prepared attaching a double-sided carbon tape to a specific aluminium stub and putting a little quantity of the selected glass powders on the upper side of the tape.

Since the FE-SEM technique needs a conductive surface to scatter the electrons and create an image, all the samples were sputtered with a layer of chromium before the analysis.

2.3.2 Compositional characterization

A qualitative measure about the composition of the glass nanoparticles has been made using the energy dispersive spectroscopy (EDS), the same microscope and samples used for the morphological characterization were used. The utilized voltage was 20 kV.



Figure 2.3.2.1: Glass powder samples inside the chamber of the FE-SEM, ready to be analysed

2.3.3 Dynamic Light Scattering

The BG particles were characterized using a dynamic light scattering (DLS) device (Anton Paar, Litesizer 500), to determine their size distribution profile and agglomeration tendency in water suspension.

A small amount of powders for each sample were put in deionized water, ultrasonicated for better dispersion, and then analysed with the following device settings:

- glass refractive index: 1.5
- absorption at wavelength 670 nm: 0.5
- water refractive index: 1.3303
- water viscosity: 0.0008903 Pa·s
- equilibration time: 10 s
- target temperature: 25 °C

2.3.4 X-ray diffraction analysis

To assess the sample structure, an X-ray diffraction (XRD) analysis was performed on selected samples by means of a X'Pert diffractometer (shown in Fig. xxx), using the Bragg Brentano camera geometry, the Cu-Ka incident radiation, a source voltage of 40 kV, a current of 30 mA, an incident wavelength λ of 1.5405 Å, a step size $\Delta(2\theta)$ of 0.02°, a counting time of 1 s per step and a analysis degree 2 θ between 10° to 70°. The obtained spectra were analysed by X'Pert HighScore program equipped with PCPDFWIN database and compared with literature results.



Figure 2.3.4.1: XRD instrument

2.3.5 FTIR analysis

In order to obtain others information about the chemical composition of the powders, FTIR analysis were performed on the glasses, using the KBr pellet method. The Thermo Scientific Nicolet iS50 FT-IR Spectrometer, equipped with OMNIC software, was used in transmission. A selected number of spectral scans of 32, a resolution of 4 cm-1 and a wavenumber range between 4000 and 525 cm-1 were selected.

To perform the analysis correctly it was necessary to create KBr/BGs tablets, the tablets were made mixing 2mg of glass with 150mg of KBr at 99% (Sigma Aldric), successively pressed for 10 minutes at 100 bar with a specific instrument (see Fig. 2.3.5.1).



Figure 2.3.5.1: Instrument used to create the tablets and tablet on the specific FTIR holder

2.3.6 Acellular bioactivity test

To examine the particles ability to mineralize and create HA crystals in vitro, S4 and SBCu4 glasses were soaked in a solution that can mimic the human plasma. This particular solution is called "simulated biologic fluid" (SBF) and is prepared in order to have the same pH and ionic concentration of that present in the inorganic part of the human plasma.

The SBF solution was prepared following the Kokubo protocol [45], reported below for the preparation of 1 liter of solution:

- 700 ml of bi-distilled water was poured in a plastic container and preheated in an incubator or on a plate to reach the temperature of 36.5 ± 1.5 °C
- a pH-meter (Crison) was used to check if the temperature was stable in a range between 36-37 °C before adding the following reagents:
 - 1. 8.035 g of NaCl at ≥99%
 - 2. 0.355 g of NaHCO₃ at ≥99.5%
 - 3. 0.225 g of KCl at ≥99%
 - 4. 0.231 of K₂HPO₄·H₂O at 99.0%
 - 5. 0.311 g of MgCl₂·6H₂O at 99.99%
 - 6. 39 ml of 1M HCl
 - 7. 0.292 g of $CaCl_2 \cdot 2H_2O$ at 96.0%
 - 8. 0.072 g of Na_2SO_4 at $\ge 99\%$
- if at this point the solution has smaller volume than 900 ml, it is necessary to add enough bi-distilled water in order to reach that volume
- always maintaining the temperature at 36.5 \pm 1.5 °C, the tris (hydroxymethyl) amminomethane (HOCH₂)₃CNH₂ (TRIS) is slowly added until a pH of 7.45 \pm 0.01 is reached
- after this process 1M HCl is added dropwise to reduce the pH to 7.42 ± 0.01 (notice that the pH value can never reach a value inferior to 7.40 ± 0.01 during this part of the process)
- at this point TRIS is added until a pH of 7.45 ± 0.01 is reached
- HCl is added dropwise to lower the pH
- the alternate addition of TRIS and HCl is carried out until all TRIS (6.118 g at =99.8% purchased from Sigma Aldrich) is completely dissolved into the solution and the final pH of 7.40 ± 0.01 at 36.5
- 100 ml of bi-distilled water is added in order to reach a volume of 1
 I.
- the prepared SBF solution could be stored in the fridge (4-6°C) for max a month.

The ratio between glass powder and SBF solution was 1:1, so that for every 100mg of powder, 100ml of solution were used.

The selected soaking periods were 1, 3, 7 and 14 days and the solution was not renewed during the tests, the samples were kept in an orbital shaker at fixed temperature (37°C) and at 1000rpm to mimic the human body temperature and fluid movement, for each time point 3 different samples were prepared to have a sort of statistical analysis of the results, pH is also measured every 2 days using the same pH-meter used for the SBF solution. At the end of the period in the orbital shaker, the glass powder was removed from the solution following a fixed procedure:

- as much as possible SBF solution was removed manually, with a pipette, trying to avoid the removal of glass nanoparticles
- some deionized water was added and the suspension (water + powder) is poured into an Falkon tube and centrifuged for 10 minutes at 5000rpm
- the water was then removed with a pipette, and the powder was let dry in an incubator at 37°C until complete drying

After this process, the powders were analysed by SEM, EDS, XRD and FTIR. [46]

2.3.7 Acetic acid tests

It is known that the bioglasses left for some time in an acid medium could lose a part of the previously present ions and consequently lose or diminish the bioactivity properties. For this test, acetic acid is been used in order to mimic the condition of the glass inside the PCL solution for the electrospinning process, prepared with acetic acid as a solvent. The process is described as follow:

- 1g of powder is dissolved in 5ml of acetic acid
- The solution is left for 1 hour under a fume hood
- The acid is now removed with a pipette
- Deionized water is added
- The solution is centrifuged for 10 minutes at 7000rpm
- The water is now removed, and the powders left to dry inside an incubator at 37°C.

XRD, FTIR, SEM, EDS, and acellular bioactivity tests (1, 3, 7 and 14 days) were performed on the nanoparticles after this treatment.



Figure 2.3.7.1: S4 and SBCu4 powders during the AA test

2.3.8 BET analysis

Thanks to a method developed by Brunauer, Emmett and Teller (BET), which exploits the physical absorption of gases into the surfaces, it is possible to evaluate the specific surface area and pore volume in the glasses. The measurement (N_2 absorption and desorption) were performed in vacuum using the analyser ASAP 2020 Plus (Micrometrics, United States). [51] [52]

2.4 Preparation of the solution for the electrospinning

The second part of this master's thesis project was focused on the optimization, and characterization of composite PCL/BGs electrospun mats. To do so, using the work done by [18] as the starting ground, various parameters, such as glass percentage, glass addition method, flow rate, needle diameter and spinning time, were adjusted, applied voltage and working distance were kept fixed.

2.4.1 Preparation of the control mat

Before starting any optimization of the composite material, was necessary to prepare a control matrix using just PCL dissolved in acetic acid to see if the addition of the glasses change, and in which way the properties of the fibers. To do so, after some bibliographic research on polymer electrospun mats [47] [48] [49] [50], the chosen protocol was the one described in Liliana Liverani and Aldo Bocaccini paper " Versatile Production of Poly(Epsilon-Caprolactone) Fibers by electrospinning Using Benign Solvents". Poly(Ecaprolactone) (PCL) with an average molar mass of 80 000 (Sigma Aldrich) was dissolved in acetic acid at 98% (AA, VWR, Germany) in a w/v% ratio of 20% (in this case 0,6g of PCL for a 3ml solution, or 1g of PCL in a 5ml one). The solution was then mixed thanks to magnetic stirrer overnight, until all PCL was dissolved, and put in US for 1 h. When ready, the electrospinning solution was transferred in a BD plastic syringe with a cross section of 0,589cm², needle 18 G x 7/8", and then spun thanks to a Starter Kit 40 KV Web (Linari srl, Italy) electrospinning device. The positive electrode was clamped directly on the syringe needle whereas



Figure 2.4.1.1: Electrospinning generator and spinning setup

the negative one was clamped on a flat, rectangular collector plate clad in an aluminium foil for conductive reasons, so that the fibers deposit on it. At the end of the electrospinning process, the fibers are deposited on this aluminium foil and can be collected. The spinning parameters were set as following:

- voltage between the two electrodes: 15kV
- working distance needle-collector: 11cm
- flowrate: 0,4 mL/h
- spinning time: 30 minutes

To calculate the flowrate in the used pump (BSP-99M Razel) is necessary to follow a precise equation:

 $Flowrate = 0,23446 \cdot pump \ selector \ number \cdot cross \ section \ of \ the \ syringe$

The flowrate is expressed in mL/h, the coefficient in cm/h and the cross section in cm^2 , whereas the pump selector number is a pure number. In this way it is easy to calculate that for the needed flowrate (0,4 mL/h) the correct number on the pump must be 2,9.

2.4.2 Optimization of glass addiction and spinning parameters

After the production of the control mat the successive step was to create a composite mat with the BG particles previously synthetized (SBCu4 and S4). Voltage, distance needle-collector and PCL/AA ratio were kept constant during all the different tests.

The preparation of the solution was similar to the control one: the PCL was dissolved in AA under constant stirring overnight, and then kept in US bath for one hour, while the glasses were added simply slowly pouring the powders in the solution, stirring them manually for one minute, with a magnetic stirrer for 5 minutes and finally again in US for 2 minutes. The addition method was chosen using reference [18] data on glass addition, plus different performed tests on manual and magnetic stirring, the US bath step was added to try to help the dispersion of the particles even more, and the time was chosen short enough not to create problems at the solution, due to the fact that the effect of US bath for longer time in the studied solution were not studied.



Figure 2.4.2.1: Electrospinning solutions with S4 and SBCu4 glasses

The changed parameters were:

- the percentage of glasses particles (w/w%) compared to PCL, 20/25/30% (for a 3mL solution respectively 0,12/0,15/0,15g)
- the flowrate 0,4/0,68/0,82/0,96mL/h (these parameters were chosen simply increasing the number of the pump selector, the first time by 2 and the others by 1 every step, from 2,9 to 6,9)
- the type of used glass
- the size of the needle 18 and 21 G.

All the experiments were carried out at room temperature, usually around 23/24°C, and similar humidity values (23-26%).

The spinning time was kept at 7 minutes for most of the samples, some samples were spun for 15 minutes in order to have thicker membranes for the mechanical tests.



Figure 2.4.2.2: PCL/SBCu4 solution during the spinning process

2.5 Electrospun fibers characterization

2.5.1 Morphological tests

Morphology and diameter of the fibers were observed by SEM analysis at FAU Erlangen-Nürnberg (FE-SEM Auriga, Carl-Zeiss, Germany).

The fibers were cut with a cutter in small pieces and then attached on specific SEM holders thanks to a carbon tape.

Before the analysis, the fibers were sputtered with a layer of gold.

The diameter of the fibers was measured with the help of ImageJ analysis software (NIH, USA).

2.5.2 EDS analysis

The compositional characterization of the fibers was made through EDS analysis.

The EDS was performed both in FAU Erlangen-Nürnberg and Politecnico di Torino on the same samples used for SEM analysis. The used microscopes were FE-SEM Auriga, Carl-Zeiss, Germany for the analysis in Erlangen and a scanning electron microscope JCM-6000Plus in Torino.

2.5.3 FTIR analysis

The FTIR analysis were performed in FAU Erlangen-Nürnberg with a Shimadzu IRAffinity-1S (Shimadzu Corp, Japan) spectrometer, shown in Fig. xxx using attenuated total reflectance (ATR), number of spectral scans 40, resolution of 40 cm⁻¹ and wavenumber range between 4000 and 400 cm⁻¹.



Figure 2.5.3.1: Spectrometer used for the FTIR analysis

2.5.4 Water contact-angle measurement

Another important parameter to measure is the wettability of the material, which is estimated measuring the contact angle (CA) between the material surface and a droplet of water on the surface itself. In this case the sample is kept straight by fixing it at the sample holders used for the acellular bioactivity tests, that will be described in the next paragraphs, a droplet of water is dropped by a needle mounted on a drop shape analyser (Krüss

DSA30, Hamburg, Germany), and the contact angle automatically measured every second in a period of 10 seconds for each tested sample.



Figure 2.5.4.1: Water contact-angle measurement setup

2.5.5 Acellular bioactivity tests

The acellular bioactivity of the fibers was evaluated immersing them into a SBF solution, prepared following the Kokubo protocol, as described before. Purity of the reagents used in FAU Erlangen-Nürnberg is now listed: NaCl at 99.0%, NaHCO₃ at 100.0%, KCl at 99.5%, K₂HPO₄·H₂O at 99.0%, MgCl₂·6H₂O at 100.5%, 1M HCl, CaCl₂·2H₂O at 101.0%, Na₂SO₄ at 99.6% and TRIS at 100%. The samples were cut and put in specific 3D printed holders, whose design and printing process will be described in the next paragraph. Once the samples were ready they were soaked in SBF solution inside some 5 mL polypropylene tubes and left in an orbital shaker (37°C) for different periods of time (1, 3, 7, 14, 19 days), measuring the pH during all acellular bioactivity tests on samples immersed up to 19 days , and never changing the soaking medium. For each time point and both glasses, the test was done in triplicate to have a sort of statistic about pH values and to have enough mats for successive testing.

The volume of the needed SBF solution for each sample was calculated using the same proportion used in Kokubo protocol, $V_s=S_{tot}/10$, where V_s is the volume of the solution expressed in mL and S_{tot} is the sample-medium contact area expressed in mm², to be noticed that in this case the inner

diameter of the sample holders was 4.8 mm so the medium volume had to be 4.7 mL.

After each time point, the samples were removed from the SBF solution, washed with distilled water and characterized through SEM, EDS, FTIR and contact angle analysis. Note that some SEM tests were performed at Politecnico di Torino (FE-SEM Jeol JCH-6000 plus) and before the analysis the fibers were sputtered with a layer of chromium.



Figure 2.5.5.1: PCL/BGs membranes mounted on the specific holders for acellular bioactivity test

2.5.6 Holders design and printing process

The usual holders dimension used for the acellular bioactivity tests in the biomaterial labs of FAU Erlangen-Nürnberg, was compatible with the 24 well plate. Due to the fact that the spinning process with the utilized solution created very small mats, it was useful to design and print new holders compatible with a 48 well plate, to try to use less material for each studied sample.

The design was made using a classic 3D CAD model design software,

Autodesk Inventor (Autodesk, CAL, USA), and after some printing test the definitive measurements were chosen as follow:

- outer ring:
 - o outer diameter: 8mm
 - o inner diameter: 6,8mm
 - ring thickness: 0,6mm
 - o hight: 4mm
- holder:
 - o upper outer diameter: 6mm
 - upper cylinder thickness: 0,6mm
 - o bottom outer diameter: 8mm
 - o inner diameter: 4,8mm
 - total hight: 8mm (4mm top, 4mm bottom)



Figure 2.5.6.1: Comparison between CAD 3D model and printed holder

After the design process the file was uploaded in the printer software (Ultimaker Cura), and after choosing the printing settings such as needle temperature (260 °C), plate temperature (100 °C), used material and infill density, the holders were printed using a polycarbonate (PC) wire in an Ultimaker S5 3D printer.



Figure 2.5.6.2: Holders printing process

2.5.7 Mechanical tests

The mechanical properties of the electrospun mats were evaluated by a uniaxial tensile test using a 50 N load cell, and testing speed of 10 mm/min. The used device was a uniaxial testing machine (INSTRON 5967), shown in Fig. 2.5.7.2.

The samples were first cut in 3x20 mm stripes and then mounted on a square paper support with inner side 10 mm and outer side 20 mm. An example of mechanical test sample is shown in Fig. 2.5.7.1.



Figure 2.5.7.1: PCL stripes before and after being mounted on the paper support for mechanical test

The thickness of the stripes was measured by a digital micrometer (precision 1 μ m) in various parts of the sample after attaching the stripes to the paper frames, and then averaged for better accuracy.

The test was performed as follow:

- the paper frame was fixed by two clamps at the machine
- the paper frame was cut to expose just the material stripe to the load
- the load was applied until sample failure

This test was performed 5 times for each sample. The tested materials were PCL, PCL/S4 and PCL/SBCu4.



Figure 2.5.7.2: Mechanical test machine during the test of a sample

3 Results

3.1 Morphological characterization

The SEM images of all synthetized glasses are reported below with comments regarding shape, aggregation, and dimension. (Fig. 3.1.1 to Fig. 3.1.16)



Figure 3.1.1: Synthesis S1_ab (S1) at 20k (a) and 100k x



Figure 3.1.2: Synthesis S1_ab_Ca3h (S2) 100k x



Figure 3.1.3: Synthesis S2_b (S3) at 20k x



Figure 3.1.4: Synthesis S2_b_Ca3h (S3.1) 20k x



Figure 3.1.5: Synthesis S2BCu_b (SBCu) at 20k x



Figure 3.1.6: Synthesis S2BCu_b_no_sol (SBCu4) at 40k (a) and 20k (b) x



Figure 3.1.7: Synthesis S2BCu_b_no_sol_cent (SBCu4 cent) at 20k (a) and 40k (b) x



Figure 3.1.8: S2BCu_b_end (S5) at 20k (a) and 200k (b) x



Figure 3.1.9: Synthesis S2BCu_b_end_cent (S5 cent) at 20k (a) and 300k (b) x



Figure 3.1.10: Synthesis S2BCu_b_end_stufa (S5 stufa) at 20k (a) and 200k (b) x



Figure 3.1.11: Synthesis S1BCu_b (S6) at 20k (a) and 40k (b) x



Figure 3.1.32: Synthesis S1BCu_b_cent (S6 cent) at 20k (a) and 40k (b) x



Figure 3.1.13: Synthesis S1BCu_b_US (S7) at 20k (a) and 40k (b) x



Figure 3.1.14: Synthesis S1BCu_b_US_cent (S7 cent) at 20k (a) and 40k (b) x



Figure 3.1.15: Synthesis S3BCu_b (S8) at 10k x



Figure 3.1.16: Synthesis S1BCu_ab_US (S9) at 20k (a) and 40k (b) x

- S1_ab: as can be easily seen in Fig. 3.1.1, the nanoparticles produced by this synthesis method are small, with an irregular shape and tend to form big aggregates, these results are coherent with the results reached by previous works [18]. The mean particle size of all glasses is smaller than 100 nm and the predominant diameter seems to be 50 nm, although it is difficult to measure the effective particle size due to particle agglomeration.
- S1_ab_Ca3h: the addition of the calcium precursor after 3 h (Fig. 3.1.2) does not lead to particular morphological changes in particles formation, if compared to the previous synthesis, even though a slight increase of diameter (50-100 nm), can be noticed
- S2_b: the synthesis process by two different solutions seems to be really effective in controlling particle shape (perfectly round particles) and reducing aggregation (no aggregation can be noticed) (Fig. 3.1.3), but leads to particles 5 to 10 times bigger than the previous methods (400-500 nm)
- S2_b_Ca3h: the addition of calcium after 3 h (Fig. 3.1.4), as already seen between synthesis S1_ab and S1_ab_Ca3h, seems to not affect particles shape or aggregation, but still leads to a slight increase in particles diameter (600 nm)
- S2BCu_b: the addition of copper and boron precursors (Fig. 3.1.5), does not lead to significative changes in particles size (700 nm), shape or aggregation

- S2BCu_b_no_sol: the removal of the liquid part of the solution before the addition of copper and boron precursors, leads to a slight particles' aggregation and formation of "sintering necks", as can be seen in Fig. 3.1.6, the shape is still spherical and the particles size (500 nm) remains constant
- S2BCu_b_no_sol_cent: the addition of the centrifugation step at the end of the process helps the particles to be less interconnected one to the others, in Fig. 3.1.7 can be noticed a decrease in the presence of sintering necks, shape and dimensions (500 nm) do no change
- S2BCu_b_end: the addition of NH₄OH at the end of the process and consequent late gelatinization of the sol (Fig. 3.1.8) leads to more aggregated and irregular shaped particles, the diameter is smaller (50 nm).
 - Centrifugation at the end of the process (S2BCu_b_end_cent Fig. 3.1.9) and longer drying times (S2BCu_b_end_stufa Fig. 3.1.10) do not significantly change the results, apart from a slight reduction of diameter in S2BCu_b_end_cent (20 nm)
- S1BCu_b: as previously said in "Materials and methods" chapter, an increase of pH could lead to bigger particles and bigger particles could lead to less aggregation, the results of this synthesis somehow confirm this thesis, in Fig. 3.1.11 and Fig. 3.1.12 (for S1BCu_b_cent) could be easily seen the diameter increase (200-300 nm), but, even though the shape of the particles is more regular than S1_ab, the aggregation is still a big problem if compared to the results reached in previous synthesis
- S1BCu_b_US: the addition of an ultrasound bath during the mixing process, does not significantly change particles diameter (200-300 nm), shape, or aggregation state (Fig. 3.1.13), even with the addition of centrifugation (S1BCu_b_US_cent Fig. 3.1.14)
- S3BCu_b: the complete removal of liquids and the drying process of particles, before the addition of copper and boron precursors (Fig. 3.1.15), do not influence particles size (500 nm), shape, or aggregation state, if compared to the results obtained in S2BCu_b_no_sol_cent, but, from the SEM image, could be noticed the presence of salts and a pure silica matrix surrounding the particles aggregates
- S1BCu_ab_US: reaching higher pH values and adding US bath during the mixing process (Fig. 3.1.16), does have very little effects in

particles size (50 nm), shape, or aggregation state, if compared to S1_ab synthesis.

According to SEM, EDS and DLS results (EDS and DLS results will be discussed in the next chapters), the best synthesis process in term of shape, aggregation and ions incorporation was chosen to be S2BCu_b_no_sol_cent, from now on called, for commodity reasons, SBCu4.

The SEM images of the control synthesis (S4) are shown in Fig. 3.1.17, the diameter was similar to SBCu4 particles (500 nm) and can be seen the presence of very few sintering necks having the results of less aggregated particles.



Figure 3.1.17: SEM image of S4 synthesis, at 20k x

The last two synthesis, SBCu4_freezer and SBCu4_lyo are shown in Fig. 3.1.18 and Fig. 3.1.19. The different drying process does not help the aggregation state of the particles, even though, freezing seems to be slightly more effective than lyophilizing in term of results on shape and aggregation.



Figure 3.1.18: SEM image of SBCu4_freezer at 20k x Figure 3.1.19: SEM image of SBCu4_lyo at 20k x

3.2 DLS analysis

From Table 3.2.1, can be seen that the results of DLS measurements are in line with the results of SEM analysis, the hydrodynamic diameter shows the mean diameter of particles aggregates whereas the polydispersity index is a measure of how much the analysed particles tend to create aggregates, lower values are a measure of lower particles aggregation [53].

The chosen synthesis S2BCu_b_no_sol_cent (SBCu4) has values of hydrodynamic diameter and polydispersity index respectively 1557 nm and 26,8%, that were considered good enough for this master's thesis porpoises, to be remembered is that the measured particles diameter in SEM images was 500 nm.

Name	Hydrodynamic diameter	Polydispersity index
S1_ab	2523 nm	45.8%
S1_ab_Ca3h	1902 nm	40%
S2_b	778 nm	42,5%
S2_b_Ca3h	549 nm	22.5%
S2BCu_b	573 nm	17%
S2BCu_b_no_sol	3193 nm	35,5%
S2BCu_b_no_sol_cent	1557 nm	26.8%
S2BCu_b_end	663 nm	30%
S2BCu_b_end_cent	785 nm	38,4%
S2BCu_b_end_stufa	3145 nm	33.7%
S1BCu_b	1470 nm	27%
S1BCu_b_cent	1410 nm	49%
S1BCu_b_US	1639 nm	32%
S1BCu_b_US_cent	3802 nm	25%
S3BCu_b	811 nm	34.7%
S1BCu_ab_US	568 nm	32.2%

Table 3.2.2: Hydrodynamic diameter and polydispersity index of different syntheses

3.3 EDS analysis

Chemical composition of BG particles and effective presence of selected ions are confirmed by EDS analysis results. Before showing the weight and atomic percentage of ions for each synthesis and spectra images, it is important to notice that:

- the presence of chromium peak is due to the fact that all the samples where sputtered with chromium before analysis
- the EDS analysis is a qualitative analysis method, so the percentage of ions presence shown could be affected by some errors or low precision
- the boron presence could not be detected by EDS analysis; thus it is considered to be present when calcium, phosphorus and copper are present in a sufficient amount, comparable with the theoretical composition
- common and demonstrated also in previous experimental studies
 [54]

S1_ab

Table 3.3.1: S1_ak	glass composition
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	Averaged w%	Average a%
Si	82,11	86,20
Р	2,95	2,81
Са	14,94	11,00



Figure 3.3.1: EDS spectrum of S1_ab glass

S1_ab_Ca3h

Table 3.3.2: S1_ab glass composition

	Averaged w%	Average a%
Si	64,98	71,72
Р	5,16	5,17
Са	29,86	23,11



Figure 3.3.2: EDS spectrum of S1_ab_Ca3h glass

S2_b

Table 3.3.3: S2_b glass composition

	Averaged w%	Average a%
Si	96,20	97,31
Р	0,00	0,00
Са	3,80	2,69



Figure 3.3.3: EDS spectrum of S2_b

S2_b_Ca3h

Table 3.3.4: S2_b_Ca3h glass composition

	Averaged w%	Averaged a%
Si	96.23	97.05
Р	1.37	1.25
Са	2.40	1.70



Figure 3.3.4: EDS spectrum of S2_b_Ca3h glass

S2BCu_b

	Averaged w%	Averaged a%
Si	92.48	94.62
Р	1.56	1.45
Са	4.68	3.35
Cu	1.28	0.58



Figure 3.3.5: EDS spectrum of S2BCu_b glass

S2BCu_b_no_sol

Table 3.3.6: S2BCu_b_no_sol glass composition

	Averaged w%	Averaged a%
Si	65.30	74.42
Р	5.36	5.54
Са	17.74	14.19
Cu	11.60	5.85



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S2BCu_b_no_sol_cent

	Averaged w%	Averaged a%	
Si	73.00	81.80	
Р	2.23	2.26	
Са	12.67	9.95	
Cu	12.10	6.00	

Table 3.3.7: S2BCu_b_no_sol_ cent glass composition



Figure 3.3.7: EDS spectrum of S2BCu_b_no_sol_cent glass

S2BCu_b_end

Table 3.3.8: S2BCu_b_end glass composition

	Averaged w%	Averaged a%	
Si	68.07	77.12	
Р	3.10	3.18	
Са	17.96	14.26	
Cu	10.87	5.45	



Figure 3.3.8: EDS spectrum of S2BCu_b_end glass

S2BCu_b_end_cent

Table 3.3.9: S2BCu_b_end_cent glass composition

	Averaged w%	Averaged a%	
Si	68.35	77.01	
Р	2.21 2.26		
Са	20.83	16.45	
Cu	8.61	4.29	



Figure 3.3.9: EDS spectrum of S2BCu_b_end_cent glass

S2BCu_b_end_stufa

	Averaged w% Averaged as			
Si	67.14	76.34		
Р	4.28	4.42		
Са	16.56	13.20		
Cu	12.02	6.04		

Table 3.3.10: S2BCu_b_end_stufa glass composition



Figure 3.3.10: EDS spectrum of S2BCu_b_end_stufa glass

S1BCu_b

Table 3.3.11: S1BCu_b glass composition

	Averaged w% Averaged as			
Si	75.87	82.75		
Р	4.63	4.57		
Са	11.58	8.85		
Cu	7.92	3.82		



Figure 3.3.11: EDS spectrum of S1BCu_b glass

S1BCu_b_cent

Table 3.3.12: S1BCu_b_cent glass composition

	Averaged w%	Averaged a%	
Si	76.10	84.69	
Р	2.07	2.09	
Са	8.61	6.72	
Cu	13.22	6.50	



Figure 3.3.12: EDS spectrum of S1BCu_b_cent glass

S1BCu_b_US

	Averaged w%	Averaged a%	
Si	77.21	83.98	
Р	2.46 2.43		
Са	13.56	10.33	
Cu	6.77	3.26	





Figure 3.3.13: EDS spectrum of S1BCu_b_US glass

S1BCu_b_US_cent

Table 3.3.14: S1BCu_b_US_cent glass composition

	Averaged w% Averaged as			
Si	82.54	88.79		
Р	1.85	1.81		
Са	7.14	5.38		
Cu	8.49	4.04		



Figure 3.3.14: EDS spectrum of S1BCu_b_cent glass

S3BCu_b

Table 3.3.15: S3BCu_b glass composition

	Averaged w%	Averaged a%	
Si	54.28	67.83	
Р	4.47	5.06	
Са	13.30	11.65	
Cu	27.96	15.46	



Figure 3.3.15: EDS spectrum of S3BCu_b glass

S1BCu_ab_US

Table 3.3.16: S1BCu ab US glass composition

	Averaged w%	Averaged a%	
Si	65.64	73.97	
Р	8.10	8.28	
Са	16.01	12.64	
Cu	10.25	5.11	



Figure 3.3.16: EDS spectrum of S1BCu_ab_US glass

All synthesis, apart from S2_b, S2_b_Ca3h and S2BCu_b (Fig. 3.3.3, Fig. 3.3.4, Fig. 3.3.5) showed a good ions incorporation compared to theorical percentages. A common pattern, that can be seen is that the centrifugation process, even though is good to avoid particles aggregation, leads to a lower ions' incorporation.

The chosen synthesis, even after chemical characterization, was still S2BCu_b_no_sol_cent (Fig. 3.3.7), because the ions percentage was still comparable with the bioactive glasses produced in previous works [18], and had a composition near to the theoretical one, that have been proven to be bioactive and non-toxic for the cells.

The results of all the synthesis kept under magnetic stirring for 24 h are not shown because they had not changes in ions incorporation from the respective solutions kept under magnetic stirring for just 1.5 h.

S4 control glass

Table 3.3.17: S4 glass composition

	Averaged w% Averaged as			
Si	87.25	90.33		
Р	1.99	1.87		
Са	10.75	7.80		



Figure 3.3.17: EDS spectrum of S4 glass

The control glass had a slight decrease in phosphorus and calcium presence if compared to SBCu4 glass, but can be considered as a limit of the utilized analysis method, that, as said in precedence, is more a qualitative than a quantitative method of analysis for ions presence.

3.4 XRD analysis

Can be easily noticed as the XRD pattern of both glasses, S4 (Fig. 3.4.1) and SBCu4 (Fig. 3.4.2), shows the typical broad halo between the 15° and 35° degree, which represent, as demonstrated in previous literature works, the amorphous phase of the glasses. [55] [56] [57] [58]



Figure 3.4.1: XRD spectrum of S4 glass before (red) and after (blue) calcination process



Figure 3.4.2: XRD spectrum of SBCu4 glass before (red) and after (blue) calcination process

The spectrum of S4 glass (Fig. 3.4.1) does not have visible peaks that could indicate the presence of a crystalline phase after calcination process, whereas if it is considered the spectrum of the glass before oven treatment some peaks could be noticed that could be principally linked to silicon oxide (SiO₂ 01-082-1555), as can be seen in Fig. 3.4.3. For what concern the spectrum of SBCu4 glass (Fig. 3.4.2) the there is an opposite situation, no clear peaks could be noticed in the spectrum of the glass before oven treatment, whereas some smaller peaks could be noticed, principally around 30° and 46°, in calcinated glasses, confirming the difficulty of creating completely amorphous glasses in case of addition of elements such as copper and boron [59]. XPert analysis have shown that those peaks could be linked to the formation of copper phosphide (Cu₃P₂ 00-047-1566), as can be seen in XPert analysis (Fig. 3.4.4), but also by the formation of calcium borate (Ca₃(BO₃)₂ 03-065-0842) as shown in Fig. 3.4.5.



Figure 3.4.3: SiO₂ peaks compared to S4 glass spectrum before calcination



Figure 3.4.4: Cu₃P₂ peaks compared to SBCu4 glass spectrum after calcination



Figure 4.4.5: Ca₃(BO₃)₂ peaks compared to SBCu4 glass spectrum after calcination

3.5 FTIR analysis

The more evident peak in S4 glass FTIR analysis (Fig. 3.5.1) is the one that can be noticed around 1200-1000 cm⁻¹ and 800 cm⁻¹, can be easily found in literature that the peaks in that area can be linked to Si-O-Si bending mode (1200 cm⁻¹), Si-OH stretching (1100 cm⁻¹), Si-O-Si stretching (1070 and 800 cm⁻¹), P-O bending (1045 and 1000 cm⁻¹).[57] [61] [62] [63] [64]

For what concern the FTIR spectrum of SBCu4 glass (Fig. 3.5.2), apart for the peaks already analysed for S4 glass, can be noticed another important peak around 1500-1300 cm⁻¹ that can be caused by B-O bond (1500-1200 cm⁻¹ stretching of BO₃ units; 1400 cm⁻¹ B-O bond, 1358 cm⁻¹ B-O stretching vibrations of BO₃ units). [65] [66] [67]



Figure 3.5.1: FTIR spectrum of S4 glass



Figure 3.5.2: FTIR spectrum of SBCu4 glass

3.6 BET analysis

An important value that can be estimated from the BET analysis, apart from the surface area of the particles, is their estimated diameter, in Table 3.6.1, are shown the data for S4 and SBCu4 glasses.

The estimated diameter can be calculated by a simple equation, found in literature [68] [69]:

$$D = \frac{6}{S * \rho}$$

Where S is the surface area expressed in m^2/g , ρ is the density of the analysed material expressed in g/cm³, and D is the estimated diameter expressed in μ m.

	Surface	area	Estimated	diameter	Sample	density
	[m²/g]		[µm]		[g/cm ³]	
S4	8.01		0.75		1	
SBCu4	2.52		2.38		1	

Table 3.6.1: results of BET analysis and calculated estimated diameter of the particles

The estimated diameter of S4 particles trough BET analysis (750 nm) is close to the one measured by SEM images (500 nm), whereas there is a sensible difference in the one of SBCu4 glasses, 2380 nm in BET analysis, against 500 nm in SEM images, this could be an ulterior confirmation of a higher aggregation for SBCu4 glasses if compared to S4 ones, result that is coherent with both SEM and DLS analysis.

3.7 Acellular bioactivity test

In this paragraph are shown the results of the acellular bioactivity test, pH variation, ions concentration and apatite formation at each time point, for both the S4 and SBCu4 glasses.

The graph of the variation of pH during the 14 days immersion time is shown in Figure 3.7.1.

It can be easily noticed that the pH does not vary in the considered timespan, and the range remains constant (7.46-7.51 for S4 and 7.39-7.45 for SBCu4), this trend is in contrast with previous literature works that showed an increase of pH values during time [64] [70] [21], one of the causes of this stationarity could be a low presence of phosphorus in both S4 and SBCu4 glasses [71] [72].

To be noticed is that some boron containing glasses tend to show a flatter trend in pH values, and in general, lower pH values if compared to other bioactive glasses, this could be linked to the buffering effect of boron. Some previous works suspected that the pH might be governed by the total sum of acid and basic ion concentration, so that boron could compensate the presence of other basic ions inside the SBF solution [73].



Figure 3.7.1: pH variation for both S4 and SBCu4 glasses

Another important parameter to keep in consideration to evaluate the bioactivity of the glasses is the increase of phosphorus and calcium ions inside the glasses, to do so, EDS test were performed, and ions concentration compared to the previous EDS results.



Figure 3.7.2: P and Ca percentage trends in S4 glass according to EDS analysis



Figure 3.7.3: P, Ca and Cu percentage trends in SBCu4 glass according to EDS analysis

In Fig. 3.7.2 is shown the variation of P and Ca ions in S4 control glass, it is evident that, after a first phase of stability, the percentage of ions increase and this could be a sign for the creation of a calcium phosphate phase, which effective presence will be further discussed with the results of XRD and FTIR analysis.

Looking at the trends for SBCu4 glass (Fig. 3.7.3), P and Ca ions increase is even more evident, in fact the percentage drastically increase, especially for P ions, after just one day of immersion time, by day 7 the presence of phosphorus is almost 6 times the initial one, compared to a x4 increase in S4 glass, while the presence of calcium is more than doubled, with a similar trend for S4 glass.

Is worth mentioning the decrease in Cu concentration immediately after the first day of soaking, copper release can be also seen by naked eye due to the typical blue colour of the SBF solution containing SBCu4 glasses (Fig. 3.7.4).



Figure 3.7.4: SBF solution after 7d soaking time of SBCu4 glass

This copper release, united to the presence of boron, could help, according to previous literature results, to increase the bioactivity of the glasses [74].

X-ray diffraction analysis results are now showed (Fig. 3.7.5 to Fig. 3.7.12) and compared.



Figure 3.7.5: XRD pattern of S4 glasses after 1d in SBF solution



Figure 3.7.6: XRD pattern of S4 glasses after 3d in SBF solution



Figure 3.7.7: XRD pattern of S4 glasses after 7d in SBF solution



Figure 3.7.8: XRD pattern of S4 glasses after 14d in SBF solution

For what concern the spectrum of the S4 nanoparticles that have been put in the SBF solution, a little peak, around the 32° degree can be noticed from day 7 (Fig. 3.7.7), confirming the EDS results in showing that before day 7 the bioactivity of this glass is very limited, if not absent. This peak, among others, is typical of hydroxyapatite crystals. [75] Due to the limited intensity of this peak, and the absence of any other clear hydroxyapatite peak, can be said that this glass is not very bioactive in general, a conclusion that is also supported visually by the SEM images that will be showed later in this paragraph.



Figure 3.7.9: XRD pattern of SBCu4 glasses after 1d in SBF solution



Figure 3.7.10: XRD pattern of SBCu4 glasses after 3d in SBF solution



Figure 3.7.11: XRD pattern of SBCu4 glasses after 7d in SBF solution



Figure 3.7.12: XRD pattern of SBCu4 glasses after 14d in SBF solution

The SBCu4 glass showed a strong bioactivity already after day 1 (Fig. 3.7.9), apart from the peak at 32°, attributed to $(2 \ 1 \ 1)$, $(1 \ 1 \ 2)$ and $(3 \ 0 \ 0)$ planes for well-crystallized hydroxyapatite (according to JCPDS (09-0432) standard), already discussed and found on S4 glasses analysis, other typical hydroxyapatite peaks can be noticed, around 26° (assigned to $(0 \ 0 \ 2)$ apatite), 46° and 50° (assigned to $(2 \ 1 \ 3)$ plane apatite) degree. For other immersion times the peaks are more and more evident, principally the peak attributed to well-crystallized hydroxyapatite for 20 values around 32° (see

Fig. 3.7.12). These results are coherent with the results obtained by the EDS analysis, that showed an increase in the presence of calcium and phosphorus; but also, by the SEM images, that showed the visual presence of crystals surrounding the nanoparticles.

Following the obtained results in XRD analysis, the FTIR tests for both glasses were performed just for 7d and 14d time points, time points chosen because both glasses showed a hydroxyapatite formation, slight for S4, and clear for SBCu4. The results are shown in Fig. 3.7.13 to Fig. 3.7.16.



Figure 3.7.13: FTIR spectrum of S4 glass after 7d in SBF solution



Figure 3.7.14: FTIR spectrum of S4 glass after 14d in SBF solution

Apart from the already discussed peaks in the "FTIR analysis" paragraph, for both S4 and SBCu4 glasses could be clearly noticed a new peak around 3500 cm⁻¹, linked to the stretching mode of hydrogen-bonded OH- ions, and some small peaks around 602 cm⁻¹, linked to the phosphate bending of



Figure 3.7.15: FTIR spectrum of SBCu4 glass after 7d in SBF solution

hydroxyapatite, these characteristic peaks could confirm hydroxyapatite formation [64] [75].



Figure 3.7.16: FTIR spectrum of SBCu4 glass after 14d in SBF solution

At this point are finally shown the SEM images of the particles for each time point to have a visual confirmation for hydroxyapatite formation.

Can be easily noticed as for S4 glasses, the formation of hydroxyapatite crystals is absent after the first day of immersion in SBF solution (Fig. 3.7.17), the only thing that can be noticed at this time step is a deposit of salts derived from the SBF solution, on the particles. after 3 days (Fig. 3.7.18) it is possible to observe few apatite crystals, which are still localized



Figure 3.7.17: SEM image of S4 particles after 1d in SBF solution 60kx



Figure 3.7.18: SEM image of S4 particles after 3d in SBF solution 50kx



Figure 3.7.19: SEM image of S4 particles after 7d in SBF solution 15kx



Figure 3.7.20: SEM image of S4 particles after 14d in SBF solution 20kx

At the 7th day can be noticed a more regular hydroxyapatite growth, as easily noticeable in Fig. 3.7.19, growth that continues up to 14 days of immersion (Fig. 3.7.20), these results are concord to the previous EDS, XRD and FTIR analysis that showed a bioactivity and a hydroxyapatite formation after day 7 in SBF solution.

For what concern the SBCu4 glass, the formation of a hydroxyapatite layer can be seen already after 1 day in SBF solution (Fig. 3.7.21) even if not yet completely interconnected with the particles, result that is reached just at day 3 (Fig. 3.7.22), confirming the results of XRD and EDS analysis. The presence of hydroxyapatite continues to grow for day 7 and day 14 timepoints (respectively Fig. 3.7.23 and Fig. 3.7.24), completely covering the particles.



Figure 3.7.21: SEM image of SBCu4 particles after 1d in SBF solution 10kx



Figure 3.7.22: SEM image of SBCu4 particles after 3d in SBF solution 60kx



Figure 3.7.23: SEM image of SBCu4 particles after 7d in SBF solution 50kx



Figure 3.7.24: SEM image of SBCu4 particles after 14d in SBF solution at 20k (a) and 51.52k (b) x

In conclusion can be said that the presence of boron and copper ions helps the bioactivity of the nanoparticles, if not in terms of final result, at least in term of reaction and hydroxyapatite formation time.

3.8 Acetic acid test

After 1 h in acetic acid, acellular bioactivity tests, XRD, FTIR, EDS and SEM analysis were performed on the powders.

The only element that had a decrease in percentage after the acetic acid treatment, was copper, trough EDS analysis has been calculated that at time point 0 (before the acellular bioactivity tests) the percentage of copper in SBCu4 glass has diminished by more than 3%, going from 6% before AA treatment to 2.85% after AA treatment. Even though P and Ca concentration remained constant after 1 h in AA, looking at the graph in Fig. 3.8.1, that shows the percentage of P and Ca ions in the S4 glass after 7 and 14 days in SBF solution, can be easily noticed that the percentage of the two ions remained constant trough time, giving a bad feedback in terms of bioactivity. Glass SBCu4, instead, even with lower percentage of Cu, showed an increase in P and Ca ions presence already after 3 days (Fig.3.8.2), confirming the results of the previous paragraph in terms of a better bioactivity for the glass with boron and copper presence.



Figure 3.8.1: P and Ca percentage trends in S4 glass after AA treatment according to EDS analysis



Figure 3.8.2: P, Ca and Cu percentage trends in SBCu4 glass after AA treatment according to EDS analysis

XRD analysis results are now showed. In Fig. 3.8.3 is represented the spectrum of S4 glasses after AA treatment, the amorphous zone between the 15° and 35° degree is still noticeable, and there are no other evident peaks that could indicate a crystalline phase in the glasses.



Figure 3.8.3: XRD spectrum of S4 glasses after AA treatment

The SBCu4 glass, instead (Fig. 3.8.4), apart from the amorphous zone, shows very evident peaks from the 11° to the 15° degree, and another one around the 27° degree, these new peaks could be due to the formation of copper acetate, as also suspected in the work done by [18].



Figure 3.8.4: XRD spectrum of SBCu4 glasses after AA treatment

Some small peaks can be seen in XRD spectrum on S4 glasses, after AA treatment and 7 days in SBF solution (Fig. 3.8.6), around 30° and 46°, and this could indicate, as written before, the presence of some hydroxyapatite crystals. Those peaks are not yet visible at the third day (Fig. 3.8.5).



Figure 3.8.5: XRD spectrum of S4 glasses after AA treatment and 3d in SBF solution



Figure 3.8.6: XRD spectrum of S4 glasses after AA treatment and 7d in SBF solution



Figure 3.8.7: XRD spectrum of S4 glasses after AA treatment and 14d in SBF solution

For what concern the SBCu4 glasses, can be easily seen the formation of typical hydroxyapatite peaks at 26°, 32°, 46° and 50° degrees, whereas the copper acetate peak disappears, probably thanks to a the washing passage with bi-distilled water, just after one day (Fig. 3.8.8).



Figure 3.8.8: XRD spectrum of SBCu4 glasses after AA treatment and 1d in SBF solution



Figure 3.8.9: XRD spectrum of SBCu4 glasses after AA treatment and 3d in SBF solution



Figure 3.8.10: XRD spectrum of SBCu4 glasses after AA treatment and 7d in SBF solution



Figure 3.8.11: XRD spectrum of SBCu4 glasses after AA treatment and 14d in SBF solution

FTIR results (Fig. 3.8.12 to Fig. 3.8.15) show no significative difference for both S4 and SBCu4 glasses after AA treatment, if compared to non-treated glasses.



Figure 3.8.12: FTIR spectrum of S4 glass after AA treatment and 7d in SBF solution



Figure 3.8.13: FTIR spectrum of S4 glass after AA treatment and 14d in SBF solution



Figure 3.8.14: FTIR spectrum of SBCu4 glass after AA treatment and 7d in SBF solution



Figure 3.8.15: FTIR spectrum of SBCu4 glass after AA treatment and 14d in SBF solution

Finally, are showed the SEM images of S4 and SBCu4 glasses, after the AA treatment, to have a visual representation of hydroxyapatite growth (Fig. 3.8.16 to Fig. 3.8.19).

For the S4 glass the only time point in which a sort of mineralization can be noticed in SEM images, the one after 7 days in SBF solution (Fig. 3.8.16), in this case would be too much talking about the formation of a hydroxyapatite layer, a better definition could be salt deposit from the SBF solution to the glasses.

A completely different statement could be done for the SBCu4 glass, that shows the formation of hydroxyapatite crystal after just 3 days in SBF solution (Fig. 3.8.17).



Figure 3.8.16: SEM image of S4 particles after AA treatment and 7d in SBF solution at 100kx



Figure 3.8.17: SEM image of SBCu4 particles after AA treatment and 3d in SBF solution at 15kx



Figure 3.8.18: SEM image of SBCu4 particles after AA treatment and 7d in SBF solution at 15kx



Figure 3.8.19: SEM image of SBCu4 particles after AA treatment and 14d in SBF solution at 20kx

In conclusion can be said that the immersion of SBCu4 nanoparticles in an acetic acid solution for 1 hour did not compromise their bioactivity properties, whereas seems to be a problem for the control particles. Further studies are needed to verify reaction of S4 particles after AA treatment due to operator guided problems in the bioactivity tests.

3.9 Optimization of the electrospinning solution

3.9.1 Fiber morphology and spinning parameter

Are now listed all the combination of utilized parameters with comments on results:

- S4 and SBCu4 glasses
 - o **20%**
 - flowrate 0.4 mL/h: the solution was easy to electrospin, very small mat created and difficult to remove from aluminium foil
 - flowrate 0.68 mL/h: the solution was easy to electrospin, small mat, easier to remove from the aluminium foil
 - flowrate 0.82 mL/h: same as the previous one, no visible difference by naked eye
 - flowrate 0.96 mL/h: no noticeable difference in the electrospinning process and mat formation, difficult to remove from the aluminium foil
 - o **25%**
 - for each flowrate there were no visible changes from the 20% glass solution in the mat formation, but worth mentioning is that some glass particles deposit could be seen in the syringe during the electrospinning process, this could lead to a lower glass amount on the PCL mat if compared to the theoretical glass percentage
 - o **30%**
 - the solution was impossible to electrospin with both 18 and 21 G needles

After a first evaluation based on spinning difficulty, size of the mats and easiness of the mat's removal from the aluminium foil, the mats produced with flowrates of 0.68 and 0.82 mL/h, both for 20 and 25% of glasses, were chosen for further evaluation and SEM analysis (as reported from Fig. 3.9.1.1 to Fig. 3.9.1.6) in order to choose which parameters were better.



Figure 3.9.1.1: SEM images of PCL fibers (0,82 mL/h) with S4 glass (20%) at 1k (a) and 10k (b) x



Figure 3.9.1.2: SEM images of PCL fibers (0,82 mL/h) with S4 glass (25%) at 1k (a) and 5k (b) x



Figure 3.9.1.3: SEM image of PCL fibers (0,68 mL/h) with S4 glass (25%) at 1k x

By the SEM images can be seen as, for S4 glasses, there were no big differences between 20 and 25% of glass, with a 0,82 mL/h flowrate. The glasses particles were incorporated in the fibers for both percentages as shown in Fig. 3.9.1.1, for the 20% solution, and Fig. 3.9.1.2 for 25%, and those, that at first impressions seemed to be breeds in the mats are proven to be, as perfectly showed in Fig. 3.9.1.2 (b), PCL fibers with some particles aggregate inside. Is worth saying that by SEM images can't be noticed a different percentage of glass incorporation between 20% and 25% mats, somehow proving, that, as previously said, glass particles deposit on the syringe for the 25% solution reduce the effective percentage of glass in the mats.

For what concern the 0,68 mL/h flowrate, in Fig. 3.9.1.3 is showed the mat produced with the 25% glass solution, there are no visible differences with the 0,82 mL/h flowrate in fibers defects or glass' incorporation. The SEM images for the SBCu4 glass show different results, with a flowrate of 0,82mL/h and a glass percentage of 20% the fibers do not present particular defects (Fig. 3.9.1.4), the mat was similar to the one produced with S4 glass (Fig. 3.9.1.1) and a good particles incorporation inside the fibers can be noticed (Fig. 3.9.1.4 (b)). But if the glass percentage was raised (Fig. 3.9.1.5) or the flowrate changed (Fig. 3.9.1.6), the mats began to show the presence of evident defects/breeds as the shape and dimensions of the fibers became irregular.



Figure 3.9.1.4: SEM images of PCL fibers (0,82 mL/h) with SBCu4 glass (20%) at 1k (a) and 5k (b) x



Figure 3.9.1.5: SEM images of PCL fibers (0,82 mL/h) with SBCu4 glass (25%) at 1k x



Figure 3.9.1.6: SEM images of PCL fibers (0,68 mL/h) with SBCu4 glass (25%) at 1k x

Following the SEM results the chosen parameters were a flowrate of 0,82mL/h and a glass percentage of 20%, the preferred needle was the bigger one, the 21 G needle, to reduce the possibility of formation of glass particle aggregates that could obstruct it avoiding the correct electrospinning process of the fibers.

After choosing the definitive spinning parameters the SEM images of both PCL/S4 glasses and PCL/SBCu4 glasses were analysed by the software ImageJ to evaluate the diameter of the fibers (Table 3.9.1.1).

Table 3.9.1.1: measured diameter of PCL fibers for S4 and SBCu4 glasses

	Diameter [µm]	Standard deviation
S4 20% 0,82	0,508	0,427
SBCu4 20% 0,82	0,409	0,335

3.10 FTIR analysis

The FTIR analysis were performed on both neat PCL fibers (Fig. 3.10.1) and composite fibers (Fig. 3.10.2, Fig. 3.10.3). It is clear that all the spectra are dominated by the PCL bands such as:

- the asymmetric and symmetric CH₂ stretching band, that could be noticed between 2943 cm⁻¹ and 2866 cm⁻¹
- the carbonyl (C=O) stretching peak clearly visible at 1722 cm⁻¹
- the C-C stretching peak at 1294 cm⁻¹
- the asymmetric and symmetric C-O-C stretching peaks, respectively, at 1240 cm⁻¹ and 1165 cm⁻¹ [47][62]



Figure 3.10.1: FTIR spectrum of PCL fibers
The absorption bands that could be linked to the bioactive glass particles are principally the silicate absorption bands which are identified by peaks at 1085 cm⁻¹, visible in both PCL/S4 and PCL/SBCu4 spectra (Fig. 3.10.2, Fig 3.10.3) even if just with a little increase in peaks already visible in the PCL spectrum (Fig. 95), 800 cm⁻¹, completely masked by PCL bands, and 464 cm⁻¹ noticeable in both composite fibers. These peaks are related to the asymmetric stretching mode, symmetric stretching vibration and rocking vibration of Si–O–Si, respectively. Peaks located at 1045 and 1090 cm⁻¹ are assigned to P-O bond but are not visible due to the presence of PCL and silicate bands. Another peak that could indicate the presence of bioactive glass particles confirming the presence of calcium due to the Si-O-Ca related bond containing non-binding oxygen, is at 950 cm⁻¹ but, again, this peak is



Figure 3.10.2: FTIR spectrum of PCL/S4 glass composite fibers



Figure 3.10.3: FTIR spectrum of PCL/SBCu4 composite fibers

also masked by PCL peaks. These results are coherent with previous literature works [63] [76] [77].

3.11 Water contact-angle measurements

Hydrophobic nature of PCL is already established, various studied shows as the water contact-angle of an electrospun PCL mat can variate from 103° [78] to 141° [79].

The results of wettability for the PCL mats produced in this master's thesis work showed a water contact angle of $109^{\circ} \pm 2^{\circ}$ compatible with previous results and completely in line with the results of Zhang et al. [80]. The introduction of BG nanoparticles, as can be seen in Fig. 3.11.1, increased the wettability of the composite mats, even if not substantially. These results were expected as previous studies showed as bioactive glass nanoparticles could increase the wettability of a hydrophobic fibrous mat [62].



Figure 3.11.1: Contact angle values and respective pictures on different electrospun fibers

3.12 Acellular bioactivity tests

During the acellular bioactivity tests pH values were constantly measured, the results (Fig. 3.12.1) showed a slight increase in pH during time. The same

considerations previously done for the results of pH values trend of the bioactive glass particles in SBF solution could be done.



Figure 3.12.1: pH values in each time point for PCL/S4 and PCL/SBCu4 composite fibers

For each time point (1, 3, 7, 14 and 19 days, from Fig 3.12.2 to Fig. 3.12.11) EDS and SEM analysis were also performed. Sadly, the non-homogeneous dispersion of the particles in the mats did not allow to see an increase of calcium or phosphorus for bigger areas. For this reason, EDS analysis were carried out in small areas where the BG particles could be easily seen.



Figure 3.12.2: SEM image of PCL/S4 composite fibers at 1.5kx, after 1 day in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.3: SEM image of PCL/S4 composite fibers at 3.4kx, after 3 days in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.4: SEM image of PCL/S4 composite fibers at 2.2kx, after 7 days in SBF solution, and the relative EDS analysis spectrum

Both EDS analysis and SEM images for PCL/S4 composite fibers showed the presence of bioactive glass particles, but it was impossible to see an increase in Ca and P presence until 19 days of immersion in SBF (Fig. 3.12.6). For all the others time points (Fig. 3.12.2, Fig. 3.12.3, Fig. 3.12.4, Fig. 3.12.5) there are no visible differences in ion concentration.

In conclusion can be said that the mats are not much bioactive, at least until day 19 of immersion, a result in line with the previous studies carried on by [18].



Figure 3.12.5: SEM image of PCL/S4 composite fibers at 2kx, after 14 days in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.6: SEM image of PCL/S4 composite fibers at 2.2kx, after 19 days in SBF solution, and the relative EDS analysis spectrum

For what concern the PCL/SBCu4 composite fibers is important to see that, already after day 3 (Fig. 3.12.8) was possible to see a variation in the EDS spectrum not linked to the formation of hydroxyapatite crystals, with the appearance of an evident chlorin peak and a small sodium peak that could indicate the deposit of salts during SBF immersion, more precisely NaCl salts from the solution to the mats, a small increase in Ca and P ions was also visible. Trend that continued also for day 7 (Fig. 3.12.9) at which was also noticeable a beginning of hydroxyapatite formation by SEM images. At day 14 (Fig. 3.12.10) the increase of Ca and P ions concentration and hydroxyapatite crystals formation was even more evident.



Figure 3.12.7: SEM image of PCL/SBCu4 composite fibers at 2.2kx, after 1 day in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.8: SEM image of PCL/SBCu4 composite fibers at 3kx, after 3 days in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.9: SEM image of PCL/SBCu4 composite fibers at 4kx, after 7 days in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.10: SEM image of PCL/SBCu4 composite fibers at 4.4kx, after 14 days in SBF solution, and the relative EDS analysis spectrum



Figure 3.12.11: SEM image of PCL/SBCu4 composite fibers at 2.4kx, after 19 days in SBF solution, and the relative EDS analysis spectrum

Is worth mentioning that the presence of Ca and P ions drastically decreased in day 19 (Fig. 3.12.11), inverting the trend, and no hydroxyapatite layer was visible by SEM images. This result could derive from different factors:

- the used solution: in fact, the spinning solution for the mats used for day 19 test was different from the one used for other time points
- the samples for day 19 were the one used for pH control; it is possible that the pH meter damaged the samples
- lower bioactive glass concentration in the tested part of the mat

FTIR analysis were performed for both PCL/S4 and PCL/SBCu4 composite fibers for each time point. The results are showed in Fig. 3.12.12 and Fig. 3.12.13.



Figure 3.12.12: FTIR analysis of PCL/S4 fibers compared for each time point of immersion in SBF solution (the green lines indicate the new peaks)



Figure 3.12.13: FTIR analysis of PCL/SBCu4 fibers compared for each time point of immersion in SBF solution (the green lines indicate the new peaks)

In comparison with the FTIR spectra of PCL/glass fibers before immersion in SBF solution, can be immediately noticed some difference starting from day 1, not only with PCL/SBCu4 fibers (Fig. 3.12.13) but also with PCL/S4 ones (Fig. 3.12.12).

- Peak around 3180 cm⁻¹ probably related to changes in the structure of the OH groups from Si-OH to Si-O-Na⁺ bonds [61]
- small peak around 1664 cm⁻¹ that could be associated to the presence of carbonated apatite or even pure apatite [64]
- small double peak around 660-643 cm⁻¹ linked to hydroxyapatite formation [61]

All the other peaks that could be linked to the formation of a hydroxyapatite layer are masked by PCL peaks and could not be noticed.



Figure 3.12.14: FTIR spectra of PCL/S4 and PCL/SBCu4 fibers after 19 days in SBF solution

Is important to notice that the FTIR spectra of PCL/glass composite fibers after 19 days in SBF solution (Fig. 3.12.14), did not show the presence of any of the new peaks showed until day 14, confirming the possible errors made for those two samples previously listed.

The last test to evaluate the acellular bioactivity of the mats was water contact-angle measurement for each time point, in Figure 3.12.15 is showed the contact angle in relation to the respective time point for both composite mats.



Figure 3.12.15: Contact angle measurement for different immersion times in SBF solution

Can be noticed as the contact angle decreases for both glasses increasing the immersion time in SBF solution, showing the hydrophilic properties of the mats if compared to neat PCL or composite fibers before the immersion in SBF solution. To be noticed is that water contact angle is not only measure for hydrophilic properties but also of the porosity of the material, that could be increased during the acellular bioactivity test.

3.13 Mechanical tests

In Table 3.13.1 are reported, among others, the Young's modulus values of neat PCL fibers and compared to PCL/glass composite fibers, as can be easily seen the mean value drastically decrease for composite fibers, apparently in contrast with literature results that showed as the addition of an inorganic filler usually increase the Young's modulus of polymers [81] [40] [82].

Both strain at break and ultimate tensile strength (UTS) decreased with the addition of glass particles, the decrease is particularly evident for the value of strain at break of PCL/S4 composite mats and for both PCL/S4 and PCL/SBCu4 if UTS is considered, the results are coherent with previous studies [63] [83] [84].

The results could be influenced by the non-homogeneous distribution of the bioactive glass nanoparticles inside the fibers, in fact the introduction of another phase leads to the formation of a weak point/defect in the material that can cause the formation of creeks and consequently lower the mechanical properties of the material.

	Young's	modulus	Strain	at	break	Ultimate	tensile
	[Mpa]		[%]			strength	(UTS)
						[MPa]	
PCL	33.21 ± 7.34		255 ± 38			3.98 ± 1.03	
PCL/S4	9.13 ± 1.84		138 ± 18			2.38 ± 0.44	
PCL/SBCu4	9.51 ± 5.48	3	240 ± 2	26		2.40 ± 1.0	4

Table 3.13.1: Mechanic properties of the different studied fibers compared

As reported in literature both neat PCL and PCL/glass composite materials showed two different trends in the stress-strain curve is observed. The first phase is the linear (elastic) phase, while the second one is a nonlinear (plastic) phase, as can be seen in Figure 3.13.1. [79]



Figure 3.13.1: Example of stress-strain curve

4 Conclusion and future works

The synthesis of bioactive particles with a good round shape and low aggregation rates, doped with boron and copper, was achieved during this master's thesis work.

It is important to highlight the role of NH₄OH in the synthesis process, higher values of pH, in fact, lead to bigger particles, and bigger particles lead to a state of less aggregation, still keeping a good ion incorporation [12]. Other important parameters that are worth mentioning are the use of a two solutions synthesis and the removal of the liquid part of it before the addition of phosphorus, copper and boron precursors, useful respectively for the synthesis of less aggregated particles and to increase the presence of P, Cu and B ions inside the particles [17] [44].

Both control (S4) and SBCu4 glasses showed a good bioactivity, both before and after acetic acid treatments, if compared to glasses with the same composition [18].

The creation of composite PCL fibers containing BGs particles was also achieved, due to the high particle diameter, residual, even if improved, aggregation state, and non-completely optimized powder addition method into the PCL/AA solution, it was difficult to obtain monodispersed particles inside the fibers, but the results could still be considered good if compared to previous works:

- the fibers containing SBCu4 glasses were bioactive, after the 7th day of immersion in SBF solution
- the mechanical properties are not improved, principally due to a residual aggregation state in the particles that could induce week points or defect in the material that could lead to creek formation, but still good if compared to previous results [18]
- the contact angle had a slight decrease when BG particles were introduced, even if not enough to reach completely hydrophilic values, further tests are needed to value if this decrease could be enough for TE applications

In conclusion can be said that main purpose of this master's thesis, to reduce the aggregation state of the particles and create composite fibers, was reached, but the material could be improved and characterized even more:

- degradation and cellular viability tests could be done in order to complete the characterization of the mats, the latter will be carried on at FAU Erlangen-Nürnberg by Liliana Liverani and the results will be soon available
- a blend with some organic materials could be done to unite the good mechanical properties of synthetic polymers to the good bioactive properties of natural polymers, this is possible thanks to the use of benign solvents, such as acetic acid or formic acid, in fibers spinning process:
 - chitosan is a natural polymer already widely used for biomedical applications thanks to its biocompatibility, biodegradability and mostly antimicrobial properties, it was also reported to improve osteogenic differentiation, protein adsorption and cell adhesion, but it lacks the mechanical properties [86] [87], that could be improved blending it with different synthetic polymers, such as PCL, this blend was already tried, with good results in different works [85] [88]
 - cellulose is a natural polymer with good biocompatibility and biodegradability, can be electrospun, promote osteogenic differentiation of stem cells [89] and has good mechanical properties [90]
 - collagen has good biocompatibility and resorbability but bad mechanical properties [91], for these reasons it is a good candidate only if crosslinked or blended with other polymers or bioactive glasses so perfect for the wanted improvement of this master's thesis composite material [92]
 - gelatin is similar to collagen, its high hydrophobicity is ideal for better dissolubility in organic solution and electrospinnability of various synthetic polymers such as PCL [93] [94].

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