THE AMBER NANO FIBERS DEVELOPMENT PROSPECTS TO EXPAND THE CAPABILITES OF TEXTILE 3D PRINTING IN THE GENERAL PROCESS OF FABRICATION METHODS

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Abstract. The amber nano fibres are bio-active composites which could be applied in three-dimensional printing. The idea of using the amber for creating a new 3D printing material, including technological composition of the innovative amber nano and micro fibres is based on the hypothesis that organic compounds of *succinite* have an effective impact on living cells, including reparative, stimulating, for sedative effects as well protective properties from electro-magnetic field. To produce amber nano fibres, bioactive components were used that were isolated from the *succinite* and tested (*in vitro*) in the scientific laboratories. Three-dimensional printing is an emerging technology, which has been initially used to design and generate three-dimensional structures mainly for transplantation therapies. This process, which can be used for a variety of polymers, is becoming an increasingly essential component of biotechnological advancements. This article discusses the foundations of this technology, namely the processes used in conventional three-dimensional printing; it also discusses tissue engineering, a discipline of which new implementations of this technology are used as bio printing. The shortcomings of tissue engineering are addressed, including the failure of existing technology to manufacture nanofiber-based constructs used in blood vessels, cartilage, artery valves, tendon, cardiac muscle valves, muscle, and cornea. From this need for nanofiber processing, electrospinning is proposed as a possible roadmap for imminent tissue engineering three-dimensional printing technologies, and finally, the latest integration of this technology with three-dimensional printing is addressed, highlighting the existing shortcomings in maintaining mandatory nano resolutions.

Keywords: amber nano fibres, electrospinning process, three-dimensional printing and tissue engineering

Introduction

It is known that mechanical behaviour of the nanofibers (Fig. 1) is estimated to be different from the bulk to microscale fibres due to the manufacturing process and large surface to volume ratio. To determine their mechanical properties is not an easy task due to very small diameter and fragility Tensile testing is the most suitable method for determining the mechanical properties of nanofibers, since nanofibers are commonly used to hold axial loads. To run the tensile and Nano indentation tests, few assumptions are needed. From the experiments, it is concluded that thinner nanofibers provided higher ductility, as well as reduced fibril stresses [1].

This process, which can be used for a variety of polymers, is becoming an increasingly essential component of biotechnological advancements. The foundations of this technology, namely the processes used in conventional three-dimensional printing; it also discusses tissue engineering, a discipline of which new implementations of this technology are used as bio printing. The shortcomings of tissue engineering are addressed, including the failure of existing technology to manufacture nanofiber-based constructs used in blood vessels, cartilage, artery valves, tendon, cardiac muscle valves, muscle, and cornea. From the existing prerequisite for the manufacture of nanofibers, potential tissue engineering developments include the introduction of electrospinning as a new pathway.

It is noted that the diameter limit showing the greatest mechanical change (250 nm) in this case overlaps the diameter limit of biological fibres such as collagen fibrils. Polymer fibres are abundant in biological materials and tissues and are commonly credited with conferring superior mechanical properties and hardness on them. For example, silk fibres are made of Nano fibrils varying in size from 25 to 170 nm with a nanofibrillar arrangement.

Ultrafine fibres have superior mechanical properties. The top-down electro spinning process produces continues nano fibres, it is very easy to handle and inexpensive to produce. In electrospinning nanofiber material properties depends on their micro and nano architecture same as elasticity of fibre complexes depends on fibre diameter and alignment distribution.

The author found that uniformly scattered discontinuous fibres help to bridge the flanks of the fracture, thus supplying some post-cracking “ductility.” If the fibres are adequately strong and bound to

the matrix substrate, the FRC can carry major stresses over a reasonably broad strain area during the post-cracking period (stage of visual crack formation and growing) [3; 4].

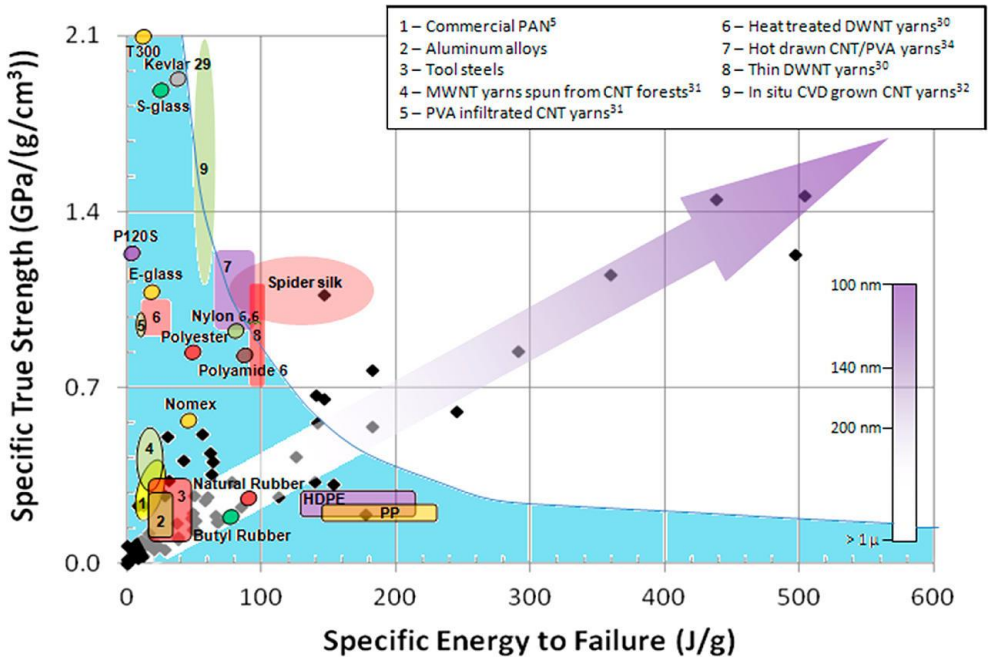

Fig. 1. **Specific intensity and specific energy to failure of as-spun nanofibers are related to values for fibres and materials [1]**

Below, in Fig. 2 represents the typical arrangement of electrospinning process. External electric force transports the silicone solvent from the syringe to the drum, and the external and internal electric forces bend and twist the charged jet into complicated forms.

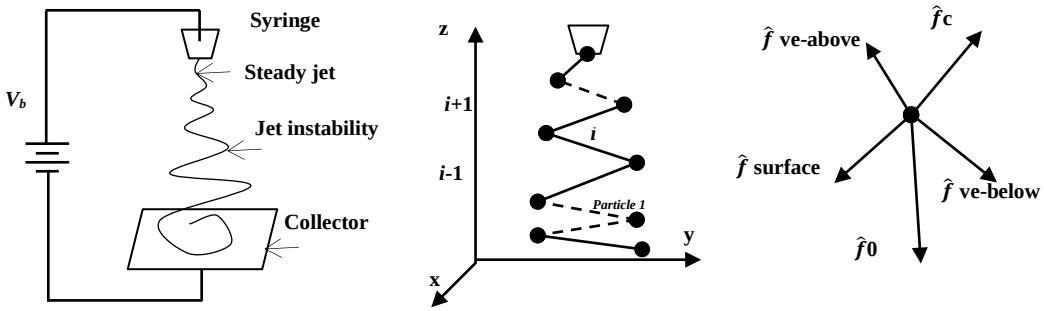

Fig. 2. **Electric spinning process and numerical notation of electro spun jets [1; 2]**

A charged jet can be described as a string of particles with charge e and mass m that are linked by viscoelastic components. The dynamic forces working on a jet particle are depicted in the diagram below [1; 2].

- Electrical force produced by an external field:

$$\hat{f_0} = -e\left[\hat{\imath}E_x(t) + \hat{\jmath}E_y(t) + \hat{k}E_z(t)\right], \quad (1)$$

where $E_x(t)$, $E_y(t)$, and $E_z(t)$ – denote the components of the electric field in the x, y, and z directions, respectively.

- Coulomb force is used to characterize particle-particle interactions:

$$\hat{f_c} = \sum_{\substack{j=1,N \\ j\neq i}} \frac{e^2}{R_{ij}^2}\left[\hat{\imath}\frac{x_i - x_j}{R_{ij}} + \hat{\jmath}\frac{y_i - y_j}{R_{ij}} + \hat{k}\frac{z_i - z_j}{R_{ij}}\right] \quad (2)$$

where i, j, and k – corresponding unit vectors along the x, y, and z axes;

$$R_{ij} = [(x_i - x_j)^2 + (y_i - y_j)^2 + (z_i - z_j)^2]$$

- Viscoelastic force:

$$\widehat{f_{ve}} = \pi a_{ui}^2 \sigma_{ui} \left[\hat{\imath} \frac{x_{i+1}-x_i}{l_{ui}} + \hat{\jmath} \frac{y_{i+1}-y_i}{l_{ui}} + \hat{k} \frac{z_{i+1}-z_i}{l_{ui}}\right] - \\ - \pi a_{di}^2 \sigma_{di} \left[\hat{\imath} \frac{x_i-x_{i-1}}{l_{di}} + \hat{\jmath} \frac{y_i-y_{i-1}}{l_{di}} + \hat{k} \frac{z_i-z_{i-1}}{l_{di}}\right] \quad (3)$$

where a_{ui} and l_{ui} – denote radius and length of connecting element between the i-th and (i + 1)-th articles;
a_{di} and l_{di} – radius and length of connecting element between i-th and (i-1)-th particles respectively

- Surface tension force:

$$\hat{f}_{surface} = -\frac{\alpha\pi(a^2)_{av}k_i}{(x_i^2+y_i^2)^{1/2}} [\hat{\imath}|x_i|sign(x_i) + \hat{\jmath}|y_i|sign(y_i)] \quad (4)$$

where α – tension coefficient on surface;
k_i – curvature of jet.

In equation (4) gravity and friction forces from the air drag are neglected to compare electrical and mechanical forces.

For the intention of creating a final structure via the additive manufacturing process This is a widely used technique, this is an increasingly growing approach which is widely used for various materials and functions several differences arise from shifts in materials (e.g., viscosity, thermal reactivity etc.). Three processes have been identified: material deposition, powder production, and liquid processing This technology offers the addition of layers and processes to create three-dimensional artefacts.

Three-dimensional printing was able to work with both synthetic and biological polymers. These structures are referred as a biomimetic, self-assembled, and organized tissues. For example, biomimicry is used here Biomimry is concerned with important features and elements of a biological system in early phase of cellular growth, some cellular components allow for microarchitecture and structural organization to arise. This is known as self-assembly where these characteristics are attributable to innate cellular modules' capacity to synthesize extracellular components, appropriating cell signals, and patterning [5-7].

In addition, tissues can be divided into smaller functional segments that are imitated by specific anatomical structures such as self-organisation of larger organs [7]. These fundamental properties of tissue engineering, along with an in-depth knowledge of the ideal biological structure in the fields of engineering, biophysics, imaging, cell biology, biomaterials and medicine, was utilized using three-dimensional printing technologies in the context of Bio printing to produce appropriate tissue [8-10]. While it is understood that this technology can produce material tissue·s^{-1}ubstance for tissue engineering, it is limited by its inability to replicate the desirable characteristics present in *vivo*.

One such feature is the construction of fibre-reinforced materials inside tissues such as blood vessels, cartilage, artery valves, tendons, cardiac skin, muscle, and corneal valves. This limitation of Bio printing has motivated researchers to develop a viable method for producing fibre. Electrospinning is one such technique that is gaining traction in additive manufacturing and tissue engineering. It is a reasonably rapid, efficient and inexpensive method for fabricating nanofibers from polymer-solvent solutions by using the electrical field, surface load and Columbic force characteristics [11-14]. Electro spun nano- and microfibers have been produced from several polymers (natural and synthetic) [15]. This process will take place with or without a controlled extrusion/feed device [16]. Since controlled systems allow greater control over the fibre properties (quality and diameter) and a higher success rate in electrospinning. Many of the following will be addressed in depth in this article as important characteristics of additive manufacturing [17-19].

The modern capabilities and mechanisms used by the technology

To consider the potential for advances in any type of three-dimensional printing, a study of the existing capabilities and processes used by the technology must be carried out.

Standardized three-dimensional printing is typically divided into three forms owing to the types of materials treated, such as filaments, particulate matter and liquids. There are several common differences of three-dimensional printing mechanisms among these classes [20; 21]. Although there are several types of processing, essentially all three-dimensional printing can be represented in the following process steps [20]:

- regulated and operated (mechanical or pneumatic) entry of the materials into the manufacturing region.
- content is processed (typically by thermal actuation) in a regulated area (if additional layers remain, this area typically becomes a previously processing region).
- if the above phases have been replicated enough to generate the final idea, it is now eligible for removal from the manufacturing center.

The removal of three-dimensional printing pieces also involves the removal of support material from the post-processing process (whether as written support systems or as raw material).

The type of procedure that can be used is constrained by the viscosity of the product. Deposition and liquid processing approaches are optimal for processing materials with a range of viscosities [16]. As complex overhanging objects are made, both deposition and droplet-based liquid processing methodologies often involve support structures. Post-processing should be used to delete it, culminating in the goal object [16]. Stereolithographic and powder processing additive manufacturing processes incorporate a powder bed/layer by layer onto a powder spreader/roller device, allowing previous layers to act as support material and necessitating less post-processing (material removal).

In comparison to other methods of additive manufacturing, material deposition in the form of extrusion techniques is somewhat imprecise. Due to this constraint, post-processing is important for the enhancement of the surface quality deposited material. In liquid manufacturing, the maximum printing resolution is possible [20]. Usually, the above three-dimensional printing processes widely used industrial materials (artificial polymers and metals). But in recent times related additive manufacturing techniques have been used in tissue engineering.

The tissue engineering's basic feature is the sequential application of cellular building blocks; tissue generation is reflected in the three-dimensional printing of the fundamentally organized introduction of object-generating content. However, it should be mentioned, that since these three-dimensional printing mechanisms (for example, fused deposition modelling, selective laser melting and inkjet printing) involve high temperatures and pressures, these techniques will require changes when used for polymers.

Bio printing in tissue engineering

Bio printing is a type of additive manufacturing in which organic materials are used to produce organic tissues in high demand in the medical sector [21-23]. The most active methods of the bio printing technologies incorporate laser-assisted printing, extrusion-based and droplet-based. These techniques are categorized as direct or indirect, with direct referring to the printing of the final organic structure and indirect referring to the construction of sacrificial models$\cdot s^{-1}$caffolds on which the content is spread and matured before being removed through post-processing [23]. Since bio printing is a type of three-dimensional printing, it is similar in terms of both fundamental process phases and the existence of technical variation to address material production requirements, including differences in the processing of macro (cell aggregates), micro (single cells) and nano (cells and protein) [24].

The discrepancies in process-enabled biomaterials, cell viability, sufficient viscosities, vertical print stability and resulting cell density are of specific importance to tissue engineering [25].

The laser bio print requires a relatively higher viscosity inks whereas inkjet methods have lower viscosity. Comparing to alternative extrusion techniques, the viscosity of the bionomical (between 30 Pa$\cdot s^{-1}$ and 106 Pa) is important to prevent unwanted product leakage from the method Ink thin ink (some extrusion printing methods, such as laser-generated column droplets, have a print resolution of 5 m) (usually unable to generate biological objects smaller than 1 precisely). 10 to 1000 m are common. Small, (106 cells$\cdot mL^{-1}$), medium (108$\cdot mL^{-1}$) and large cell densities (for 3D-printed spheroids) are suitable with inkjet, laser-assisted and extrusion. Extrusion techniques result in lower cell viability where extrusion volume and nozzle size influence cell survival. It has been proposed that the droplet

size will range from 1 pL to 300 pL with a printing rate of 10,000 droplets per second, enabling the production of long or thin lines up to 50 μ [26].

Both inkjet and extrusion bio printing methods are restricted by the accumulating mechanism, namely of the nozzle scale, nozzle clogging (via fluid alleviation and aggregation), spatial precision, and the stress exerted on the material by shear [27-29]. Currently accessible bio printing techniques depend highly on the exact position of a cell or group of cells. The most of these three-dimensional printing techniques are constrained by the prevalent reliance on the usage of heat and pressure to achieve higher resolution (apart from electro hydrodynamic printing [30]), which may cause problems and damage to the biological material (known as denaturing).

An understanding the latest technologies for the generation of biological structures is not adequate for tissue replication. And there is a lack of three-dimensional printing and bio printing methodologies for the development of fibres based on constructs that can be used in biological structures such as blood vessels, cartilage, artery valves, tendons, cardiac tissue, muscle and cornea valves. These shortcomings show necessity for further analysis of the development a biopolymer in a pleasant type of nano fibres processing based on additive manufacturing technology.

Melt extrusion is not needed, allowing for heat-sensitive polymers and proteins to be processed. Compared to template synthesis or self-assembly, electrospinning is easier and there may allow less space for error. Easier processes, such as partitioning (which is linear) or drawing (which is uncontrolled), are better despite certain difficulties, the outlook seems to be promising for using of electrospinning nano fibres for tissue engineering.

Electrospinning material, toxicity and limitations

The standard electrospinning method is close to that of some three-dimensional printing techniques, in that both methods involve the mechanical actuation of the material at the point of manufacturing. The techniques differ in that, rather than thermal actuation, high-voltage penetration (typically greater than 20 kV) results in substrate malleability and movement/deposition [31]. In conventional electrospinning, a single nozzle-charged extruder produces a single jet·s^{-1}tream of material.

As in the three-dimensional printing technology, the methods used in this process differ according to the characteristics of mutually the material being produced and the desired effect. The essence of solvent toxicity and alignment desirability are two influential characteristics that result in heterogeneity.

Since the solvents used in this process are acidic, they can cause biocompatibility issues due to their toxicity. Melt electrospinning is a form of electrospinning that utilizes heat to melt polymers, allowing for electrospinning with a lower concern for solvent toxicity. However, this is a relatively new method (approximately 100 reported articles) that is currently capable of generating much larger fibres than those generated by conventional electrospinning [32]. This approach is focused on using of incredibly high temperatures, which may be harmful to biological content and is thus extremely unlikely to be relevant in the field of biotechnology.

This technique takes advantage of the electrostatic attraction between nano fibres with oppositely charged areas, extending the fibres between the electrodes [32]. It is worth noting that this process is relatively scale-limited (relative alignment lost at gaps larger than 30 mm [33-38]). A recent research [36] demonstrated that a mixture of alignment mechanisms could partially overcome these spatial constraints. Alagille was used in this analysis utilizing a mixture of ceramic magnets, parallel copper electrodes and filtered water.

The direct electrospinning is another technique that allows much greater control of scattering fibres. This control is obtained from the automation of the collector or extruder at close collection distances. These shorter distances allow for a decrease in randomized jet volatility, but at the cost of decreased evaporation, which results in wet electrospinning [39].

The construction of the resultant object is one of the key differences between three-dimensional printing and electrospinning. Once recycled printed material has been added, it can be easily modified, which is not possible for the mats made of electrospinning fibres. Additionally, functionalization methods are used to create external crosslinks and linkages between the fibres to strengthen the connection and facilitate manipulation. This has happened because of adaptation to steam bath technologies and simplified implementation methodologies. Historically, this incorporation of agents

occurred as a separate step from the electrospinning method, however modern advances have enabled the process to be more streamlined by using three-dimensional printing forms.

At present, electrospinning is restricted to the manufacturing of place mats (two dimensional objects). Owing to the deposition of charge from electrically spun fibres on the collection surface, the resulting material is limited to a thickness of approximately 3-4 mm [40]. Additionally, electrospinning limitations include the dangerous quality of the solvents used, efficiency/productivity and fibre power generation [41-43]. The combination of this method and three-dimensional printing was studied to address these drawbacks as well as the functionality criteria of electrospinning science. Both extrusion and droplet based on three-dimensional printing of additive manufacturing techniques were used alongside electrospinning, usually in the shape of a sheet in one process followed by a layer of the other process. This allows the development of three-dimensional structures with enhanced functionality [36-38].

Fabrication example of the amber nano fibres: materials and methods

One example of the electrospinning the amber nano fibres that can be adapted for three-dimension printing is polymer structures reinforcing with organic compounds. The principle of fabrication of the amber nano fibres is considered. But one should be noted that for tissue engineering (as well as for all implanted materials), it is permissible to use 100% of synthesized compounds. The active ingredients isolated from amber, which can be purified and approved by pharmacology, can be the basis for their subsequent productions and implementation in blood vessels implant structure [44-46].

The materials and amber nano fibres fabrication include: Developed method for fractionation of *succinite* powder (particle size 5 nm-3 μm), (CAS: 9000-02-6 EC: 232-520-0, RTU, LV) with PA6 (CAS: 25038-54-4, Sigma-Aldrich AB, Sweden) based on formic acid solution (CAS): 64-18-6, Sigma-Aldrich AB, Sweden). Ingredient ratios: 6.1496 g (HCOOH): 1.22992 g (PA6): 0.01222992 g (*succinite*).

Ingredients were weighed with analytical balance Mettler Toledo. Containers (DWK Life Sciences Wheaton ™ Glass 20mL Scintillation Vials) with suspension for homogenization were heated for 5 hours (Thermo Scientific ™ Cimarec + ™ Stirring Hotplates Series) with magnetrons (temp. + 22° C, 380 RPM).

Subsequently, the suspension container was placed in an ultrasonic bath (Branson Ultrasonics ™ CPXH Series Ultrasonic Cleaning Bath) for 30 min, the operation was repeated up to 3 times until the sample was found to contain 95% nano-sized particles. After every 30 min. a particle size control was performed using a granulometry ((Mastersizer3000) to determine particle size and percentage of different particle size). The particle structure was determined under a microscope (Quanta 200 FEG SEM).

Amber nano fibres (PA6) are electro spun at ambient temperatures using 20 Gauge needle from a 16-26% wt/wt solution of the polymer (Pfalz and Bauer, MW 10 000). On a stationary mark, fibres were gathered. The applied voltage: 12-16,5 kV, the flow rate: 0.6-1 ml/h, the spinneret and collector were 20 cm apart. The diameters of the fibres were varied by changing of the voltage and the PA6 concentration. The same electrospinning parameters were used to treat both spun and annealed fibres (Fig. 3).

The individual fibres were manually measured in the mechanical measuring device (NANO-UTM) using a strain rate of 0.001 s^{-1}. The electrically spun length of the nano fibre was used 4-5 cm. The single fibres were individually collected with a “fork”. About 5-10 mm portion of the fibre was added to the epoxy grips (the duration of the gage in this study). The adjoining fibre was examined with the Quanta 200 FEG (FEI). The diameter was tested at least ten times to make sure it was right. Fibres demonstrated plasticity, with substantial deformation at failure. The load and displacement calculations have been translated to engineering and real strain values and stress-strain diagrams. Additionally, true stress and strain represent behaviour of the tested material by the stress-strain diagrams, nano factor modulus, failure pressure and failure area were derived. The module and tension rely on graphs. The amount of as spun Nano forms screened was 52.

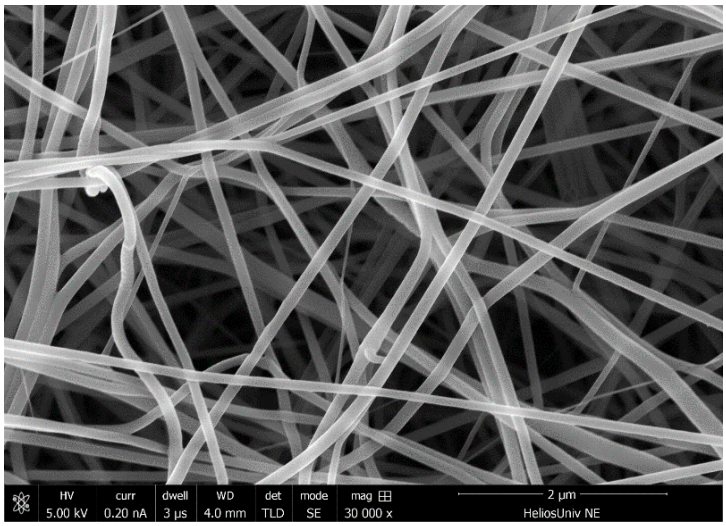

Fig. 3. **The amber nano fibres mats (University of Nebraska-Lincoln, Lincoln NE USA)**

Reducing of the amber nano fibres diameter from 2.8 μm till 100 nm shown next progressive changes of mechanical characteristics:

Elastic modulus 0.36 → 48 GPa; Toughness 0,25 → 605 MPa; Strength 15 → 1750 MPa.

At this stage, two ways of introducing nano fibres (Fig. 4) onto the structure of microfibers and biological materials are considered:

- piezoelectric inkjet printing directly on the microfibers surface [1; 2].
- piezoelectric inkjet printing directly on biological material surface.

The mechanical testing of the amber nano fibres and some technological features using of piezoelectric technology were made based on cooperation between Riga Technical University (Riga, Latvia) and University of Nebraska-Lincoln Department of Mechanical and Materials Engineering (Lincoln NE USA).

This technology can be very promising to produce biomaterials with hydrophobic properties (for example, to produce blood vessel implants). In this case, on the inner surface of the implant, in the presence of such type of amber nano fibres, the Lotus effect is created (that is, drops of liquid, in this case, blood, roll down along the surface of the implant and do not stick to the wall). This novelty effect is enhanced by the fact that amber nano fibres have an electronegative charge, and blood cells are positively charged. As well, reducing of the amber nano fibres diameter from 2.8 μm till 100 nm showed a sharp increase of main mechanical characteristics, like Elastic modulus; Toughness; Strength. A similar trend is observed with other types of polymers.

The ways of using amber nano fibres in three-dimensional printing for tissue engineering are still under the development.

Conclusion

1. A new additive manufacturing technology has been extensively applied as a method of micron-scale biotechnology for scaffolding and tissue manufacturing. Current shortcomings of bio printing include the failure to manufacture complicated fibre-based materials, such as blood vessels.
2. Additional technology has been explored to produce these microns and nano-based fibres, a promising method to do it by electrospinning. This process is close to additive processing in that it is a repository technology. Current research has a set-in motion a mixture of previously developed three-dimensional printing technologies and electrospinning methodologies for the creation of fibre-based structures.
3. Furthermore, using of the nano fibres in biopolymers is very promising. One of the most important benefits being a major improvement in the strength properties of a small nano

fibre diameter. This paper established a strong basis for future studies for the development of additive manufacturing in tissue engineering.

4. This research novelty concludes hybrid combination of electrospinning staples nanofibers and 3D printing.

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