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Basic hydrogel polymer materials for 3D printing

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Abstract: Currently, 3D printing of hydrogel scaffolds, which are used in tissue engineering to come to the fore, is gaining prominence in order to restore the structure and function of soft tissues and organs. For successful printing of soft constructs, the FRESH method using a support bath is used, thanks to which the printed hydrogel is kept in the desired shape during solidification. The aim of the study is to create an overview of hydrogel materials and their properties affecting printing, to summarize previous printing of hydrogel scaffolds. The choice of material, the method of crosslinking for the formation of the hydrogel, is taken into account, while at the same time the non-toxicity and compatibility of the material with the biological environment. Specific emphasis is placed on adapting the technological procedure of the FRESH method, where the chemical composition of the support bath must be in accordance with the crosslinking agent and the rheological properties of the printed hydrogel.

1 Introduction

Polymers are large molecules (macromolecules) made up of long chains or cycles formed by regularly repeating parts, called building blocks or monomer units. Their number indicates the degree of polymerization n, which has a value of 10 to 106 [1].

Monomers are simple molecules that undergo a polymerization process to form polymers. Therefore, monomers are also called polymer building blocks [1].

Polymerization is the process by which two or more molecules react and combine to form polymers - long chains of monomer repeat units.

Polymers can be divided into two categories according to the source and origin of the polymer:

1. Natural polymers.
2. Synthetic polymers [2].

Natural polymers - also called biopolymers - perform key functions in organisms such as building and structural proteins and polysaccharides, nucleic acids, energy supply and storage. Natural polymers include silk, rubber, cellulose, wool, keratin, collagen, starch, DNA and nucleic acids [3].

Synthetic polymers are prepared by a chemical reaction, often in a laboratory. A wide range of physical and chemical properties can be achieved on the basis of monomeric units, polymerization reactions and copolymer formation from different components in adjustable concentrations. PVC (polyvinyl chloride), polystyrene, synthetic rubber, silicone and others are basic examples of synthetic polymers [4].

A common feature of synthetic polymers is their stability, which is often required in everyday life and the medical field. The disadvantage of this stability is that these polymers cannot decompose naturally, leading to accumulation in the environment. This can cause various toxic effects in the environment. One way to decompose or destroy these synthetic polymers is to burn them or heat them to very high temperatures, which is also not an environmental approach due to the release of toxic gases [5,6].

According to the arrangement of the polymer molecules in space, they are classified as linear polymers, branched chain polymers and spatially crosslinked polymers (Figure 1).

1. Linear polymers: The basic building blocks have monomers arranged in one row, they are produced using

straight and long chains of monomers joined by a single bond. For example, polyester, nylon, Teflon, etc.

2. Branched chain polymers: They are formed by the interconnection of some parts of linear chains by chemical cross-links. They represent polymers that branch in a plane. For example, polyethylene, glycogen, starch, etc.

3. Spatially crosslinked polymers: These polymers are formed when building blocks bond to a three-dimensional network through strong covalent bonds. For example fiberglass, adhesives, polyester, etc. [7].

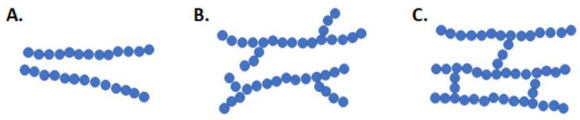

Figure 1 Scheme of polymer structures: A. linear, B. branched, C. spatially crosslinked [7]

Polymeric substances can be formed in various ways (see Figure 2) and are based on the formation:

1. Polymers formed by polymerization: Polymerization can be defined as a multiple addition reaction in which no by-products are formed. The molecules of the substance entering the reaction are gradually (multiple) combined into larger units, the resulting product being a monomer with multiple bonds.

2. Polymers formed by polycondensation: Polycondensation is a multiple reaction in which at least 2 different monomers react and the result is the removal of small molecules such as water, ammonia and the like. Reactions are always reversible and have a stepwise course, so the addition of a substance creates a new step with a new product and reaction and can be stopped at any time by the absence of a reactant. The reactions use catalysts which affect the course of the reaction. The products are generally called polycondensates.

3. Polymers formed by polyaddition: Polyaddition is the transition between polymerization and polycondensation. The reactions tend to be chain-linked, re-unsaturated monomers, and no by-product is formed [7].

Polymers are used for various purposes depending on their flexibility, tensile strength and the like. The mechanical strengths of polymers are determined by hydrogen, ionic or covalent bonds.

Based on intermolecular forces, polymers can be divided into the following categories:

1. Elastomers: Elastomers are solids with elastic properties. These polymers have weak molecular bonds between the monomers, which helps the monomers to stretch easily. For example, polybutadiene, polyisoprene, etc.

2. Fibers: They are made of chains of similar structures, which facilitates their interconnection and have high tensile strength. For example, terylene [7].

3. Thermosets are made of a liquid or soft solid which, when cured by heat or radiation, forms an insoluble, irreversible polymer, i.e., the thermosets form irreversible chemical bonds. Thermosets tend to be rigid and have a high molecular weight. Examples of thermosets are epoxy resin, polyester, acrylic resin, polyurethane, rubbers and vinyl esters.

4. Thermoplastics are solid in the cold but are flexible and malleable when heated above a certain temperature. While thermosets form irreversible chemical bonds, thermoplastic bonds are weakened by rising temperatures. While thermosets decompose and do not melt, thermoplastics melt into a liquid state by heating. Examples of thermoplastics include nylon, Teflon, polypropylene, polycarbonate and polyethylene [4].

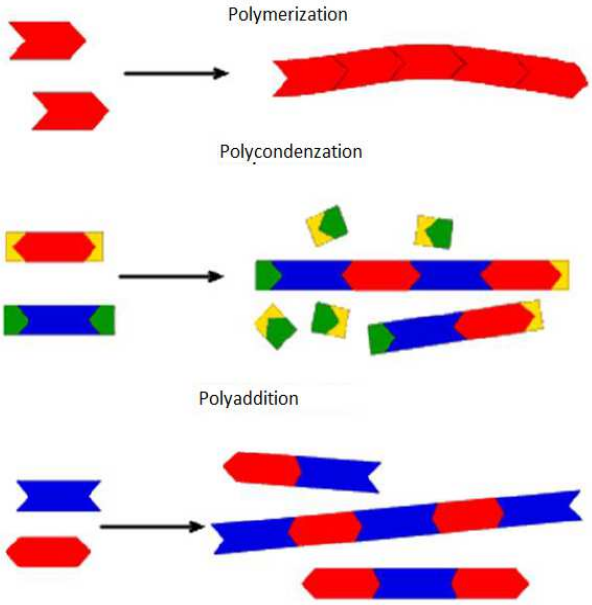

Figure 2 Types of polymer synthesis

1.1 Biodegradable polymers

A special group of polymeric substances is defined by a property specifically monitored for environmental reasons, and at the same time it is an important condition in the field of biomedical development of materials. Due to their strong bonds, a large number of polymers are very difficult to break and thus degrade. This causes the accumulation of non-degradable polymers in the environment, causes an imbalance in nature and can become toxic to the environment, living organisms as well as humans. In order to prevent the toxic effects of polymeric materials on the biological environment, emphasis is placed on testing and verification of the so-called biological tolerance and biodegradability. Such materials behave harmlessly in the biological environment and may be capable of degradation, while their degradation intermediates do not cause toxic reactions to living organisms [6].

1.2 Hydrogels as polymeric materials

The excellent mechanical and water of the acknowledging properties are a characteristic feature of hydrogel, which are based on synthetic polymers, but also have major disadvantages, namely poor biological degradation and potential toxicity, which significantly narrows the profile of their biomedical applications (see Table 1) [8].

Table 1 Summary of properties of natural and synthetic polymers

	Advantages	**Disadvantages**
Natural polymers	In natural organisms	Limited sources
	Easily degradable	Low mechanical properties
Synthetic polymers	Reproducibility	Emphasis on biocompatibility
	Artificially created	Lower degradability
	Good mechanical properties	Products of degradation could be toxic
	Customization of properties	Could need toxic polymerization solutions

Compared to synthetic polymers, hydrogels based on natural polymers have great potential for application in biomedical areas due to their biocompatibility and biodegradability. A natural polymer is a high molecular weight compound that has a linear long chain formed by repeating units as a basic structure, commonly found in animals, plants and organisms such as collagen, chitin, alginate, cellulose, starch, agar and the like [3].

In practical applications, hydrogels must carry their own weight without breaking, and in addition they must withstand a certain external force from the environment in which they are placed. The hydrogel based on natural polymer generally has poor mechanical properties, which considerably limits its application possibilities. Therefore, it is very important to improve the mechanical properties of hydrogels based on natural polymers, while paying attention to the conditions of its subsequent application, which must remain non-toxic and biocompatible [8].

2 Properties of hydrogels

2.1 Biological properties

2.1.1 Cytocompatibility

For tissue engineering, cytocompatibility is a crucial issue in selecting a applicable biomaterial. ECM-derived hydrogels naturally have cytocompatibility assets. More all-important, the numerous binding sites in hydrogel materials can allow for synergy between cells and the hydrogel matrix. Polysaccharides and synthetic hydrogels may also be compatible with cells but lack bioactive sites for cell connection and attachment. Further modification is needed to simulate the physiological microenvironment of the tissues [9].

2.1.2 Biodegradability

The ability of implants to be gradually broken down and washed out of the body by means of their biodegradability is essential in some clinical transplants. In addition, many experiments have shown that biodegradability is also crucial at the cellular level. In one study, a cell-degradable hydrogel was shown to encourage the deposition of encapsulated chondrocytes in the extracellular matrix. In another study, cells cultured in hydrogels with a more rapid rate of degradation exhibited increased migration and proliferation. Such a process is shown in Figure 3. Biodegradability offers that the mild degradation process and the degradation and degradation products of biomaterials have small effect on cell survival [10,11].

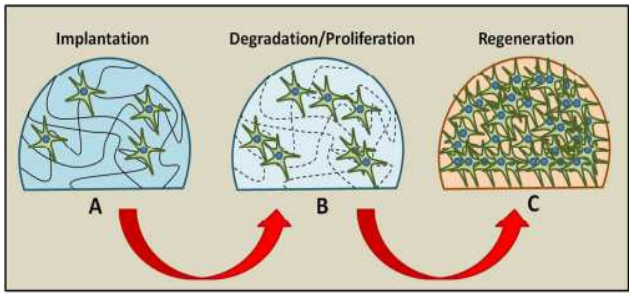

Figure 3 The ideal pathway to hydrogel mediated tissue regeneration [12]

2.1.3 Transport environment

For enduring tissue functions, mass transport of biological and chemical molecules between cells and tissues is indispacable. Hydrogels are matrices with interconnecting pores and large amounts of water. This property allow the wide distribution of small soluble molecules. In hydrogel-based tissues, biochemical factors such as gas, nutrients, proteins, metabolic wastes, etc. can be well transported across vascular boundary and through conditioned tissues [9].

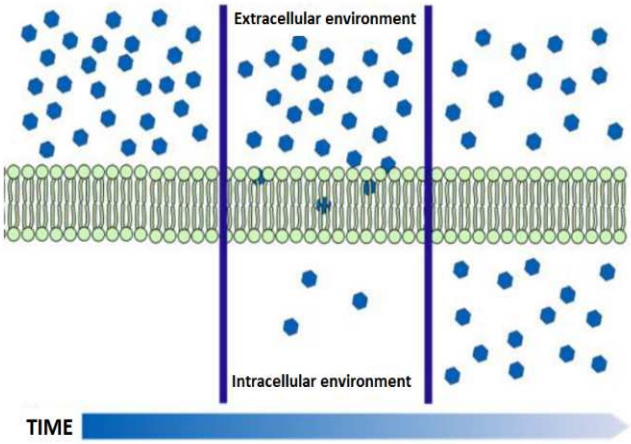

Figure 4 The ideal pathway to hydrogel mediated tissue regeneration [12]

The chemical gradient (*see Figure 4*) created during in vivo bulk transport is important as it affects cell migration, which is associated with diverse physiological and pathological mechanisms such as inflammation, embryonic morphogenesis, cancer metastases, etc. Chemicals that diffuse within the hydrogel also create a diffusion gradient in the hydrogel, and this can be used to search physiological and pathological phenomena in vitro, which so far suggest a higher importance of the chemical gradient at the tissue level than at the level of neighbouring cells in contact [12].

2.1.4 Rheological properties of hydrogels

The Department of Rheology studies the deformation and flow of materials depending on the applied force. In the extrusion-based process, the ink is initially in the storage vessel at rest (no flow). When forces are applied, it undergoes deformation and flows under high shear conditions as it moves through the nozzle walls. It then takes on a new shape until it finally returns to a new state of rest [14,15].

Viscosity, viscoelastic modulus in shear, material recovery behaviour, and shear stress are crucial rheological characteristics associated with these transitions. It may be associated with ink performance before (rest and flow initiation), during (flow behaviour - extrudability). and after printing (printing accuracy, form fidelity and adhesion), see Figure 5.

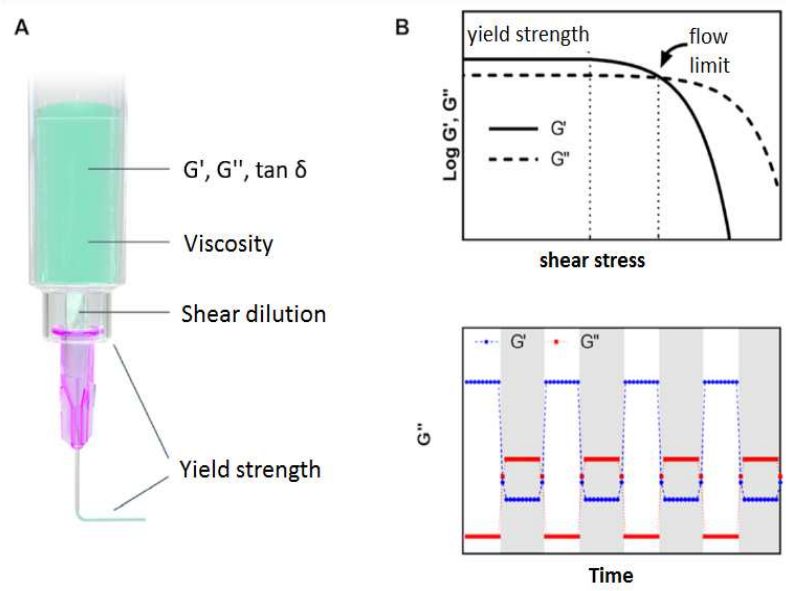

Figure 5 Rheological properties during the printing process (G 'and G' 'denote sol-gel phases and Tan δ represents the damping factor) [14]

3 3D printing of hydrogels

Overview of 3DP methods for hydrogel materials

In general, printing materials must meet the requirements of:

1. have an adequate viscosity to allow printing and structural stability,
2. be able to create a 3D structure in a few minutes,
3. have satisfactory mechanical properties,
4. be biocompatible,
5. have adequate degradation kinetics,
6. create non-toxic by-products of degradation,
7. be biomimetic,
8. be able to control the release of molecules or drugs [16].

Moreover, bio-inks should be easy to produce and process, be affordable and commercially available [16].

Scaffolds are structures that are specifically designed to mimic the extracellular matrix (ECM) of living tissue, simulate the in vivo environment, and allow for desirable cellular interactions that contribute to the formation of new functional [11,17,18]. The constructed scaffolds are used as a supportive environment in which the cells are "deployed" and the scaffolds thus support the formation of three-dimensional tissue. Scaffolds usually serve at least one of the following purposes:

- allow cells to attach and migrate,
- supply and retain cells and biochemical factors,
- allow the diffusion of vital cellular nutrients and expressed products,
- exert certain mechanical and biological effects on modifying the behaviour of the cell phase [17,19,20].

The scaffold design for biomedical applications should meet the needs of some of the basic requirements schematically illustrated in Figure 6:

1. high porosity (preferably 100% interconnection for optimal nutrient flow and tissue overgrowth by cells);
2. relevant geometry and pore dimensions (5-10 times the cell diameter);
3. biodegradable with adjusted (or modifiable) degradation time;
4. maintaining mechanical integrity for a specified period of time;
5. should have appropriate cell-biomaterial interactions; and
6. should be easy to manufacture and reproducible [11,17,20].

Bioinks or bioarmaments are materials used for the production of constructed / artificial living tissues using 3D printing. These inks usually consist of biomaterials that contain or encapsulate cells or are capable of adhering cells to the surface of the biomaterial. The combination of cells and biopolymer hydrogels is defined as bionic [14,21].

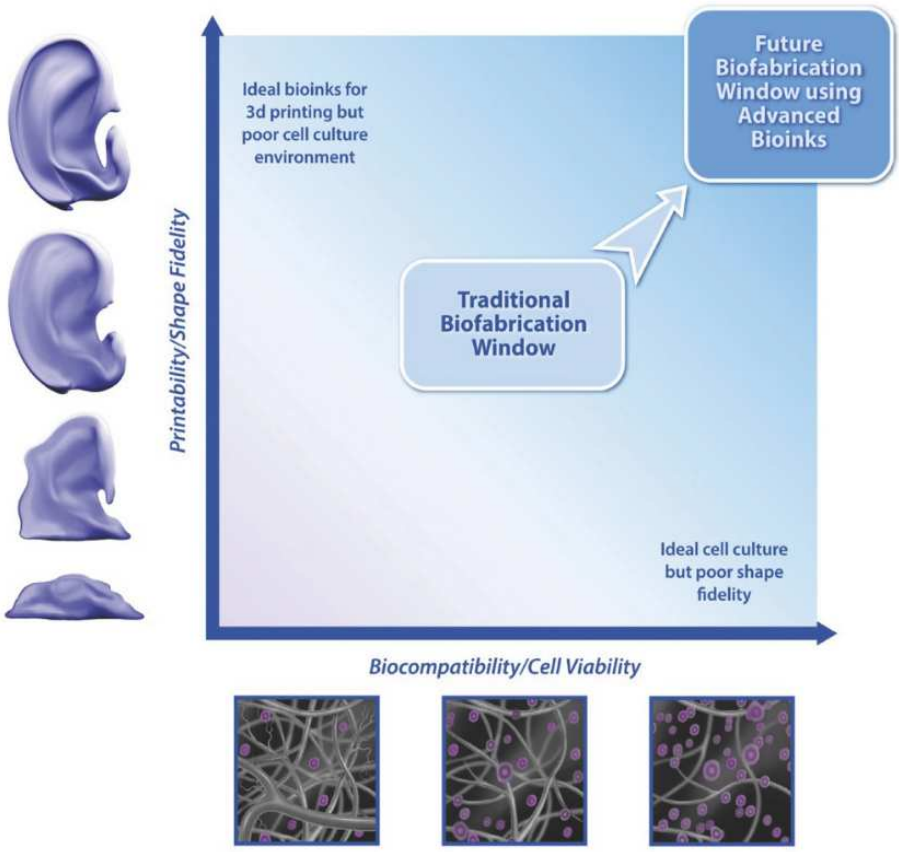

Figure 6 The biofabrication window [21]

3.1 3D printing methods of hydrogels without supporting structure

A very relevant characteristic of each technique will be its resolution. Each technique has its limit of the smallest details that can be produced. This limit represents the extent to which the planned object is feasible while maintaining the required level of detail [23].

As not all rapid prototyping (RP) techniques are applicable to the processing of hydrogel materials, this requirement has redistributed a number of RP technologies. In addition, the production of hydrogel scaffolds requires mild processing conditions, which again eliminates some technologies unsuitable for hydrogels. This chapter provides an overview of the most important techniques developed for 3D printing of hydrogel materials, including their advantages, disadvantages and limitations, which have emerged in previous studies [24].

3.2 Methods of 3D printing of hydrogels with supporting structure

As 3D printing runs layer by layer, many 3D printing materials undergo solidification of the soft material to hard, allowing previous structures to support the weight of the next layer as well as their own weight. However, hydrogels often remain deformable even after crosslinking [25,26].

Insufficient support for previously printed layers can cause the material of the previous layers to fold when the layer is added under the influence of its own weight. This affects the print quality of the final structure and is one of the most common causes of three-dimensional scaffold design failure [23]

Although it is common that even relatively rigid gels can show considerable softening during printing, this phenomenon is particularly pronounced for very soft constructions, which are of interest for applications mimicking the mechanical properties of many biological soft tissues [23,27].

Biological hydrogels are difficult for 3D printing because they must be supported during the gelling process so that they do not collapse and deform due to their own weight. The need for support materials is common in many AM techniques, yet it is particularly difficult to prevent damage to materials and potentially integrated cells for such soft materials where the modulus of elasticity is less than 100 kPa (see Figure 7). In addition, for hydrogels, there is a narrow range of mechanical, thermal and chemical conditions that must be met to maintain the shape of the hydrogel skeletons [20,25].

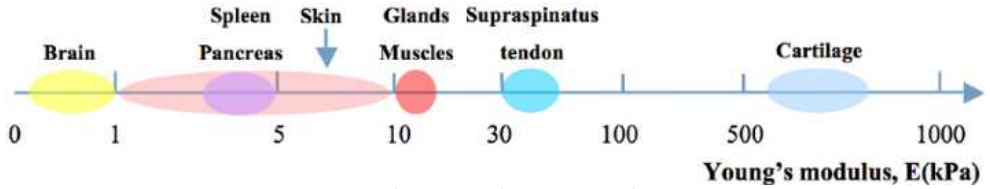

Figure 7 Young's modulus of elasticity for natural soft tissues and organs [20]

3.3 Key factors affecting the press

The most important characteristic for 3 D printing is printability. This ability to create and maintain reproducible 3D ink scaffolds using 3D printing techniques affects the structure of printed spacesuits and consequently acts on their mechanical and biological properties [28].

Structures for use as support structures for living cells can be made using extrusion-based printing techniques. For this purpose, hydrogels for their environment favourable to cells and high water content are widely used. Hydrogels can be crosslinked physically or chemically, which affects the quality of the extruded 3D structure. Crosslinking hydrogels is a time consuming process and the hydrogel as such may be dimensionally stable as a solid or flow as a liquid, thereby deviating substantially from the desired design. Due to the poor compressibility of hydrogels, printed scaffolds can sometimes collapse and do not form a 3D structure [28].

The concept of printing is important because the difference between a printed scaffold and the ideal design required can affect mechanical and biological properties, including mechanical stiffness and cellular functions [28].

Naghieh et al. divided the factors influencing printing into three categories: scaffold design, ink, and the printing process. Scaffold design parameters that may affect printability include pore size, filament orientation, and layer thickness. The critical factor associated with an ink is its flow behaviour and physical properties. Relevant parameters of the printing process include the mechanism of crosslinking (polymerisation) and the parameters of the printing itself, such as pressure and speed [28].

3.3.1 Scaffold design

Factors associated with scaffold design that affect printing include fiber orientation, fiber spacing, pore size, and layer thickness. However, a limited number of studies have identified key elements that play an important role in printability in terms of scaffold design [28].

Some studies have looked at the effect of fiber orientation during printing on the surface and overall porosity of the scaffold, cf. Figure 8. For example, changing the orientation of each fiber near the edge by 45 ° instead of 90 ° can reduce the amount of ink required. Structures with larger inner pores require less ink, on the other hand they endanger the stability of the structure [28] [29].

The thickness of the layer can affect the pore size, and by changing the layer thickness during printing, more accurate pore sizes can be achieved. The main reason for the change in layer thickness during the printing process is that the pore size does not remain constant from top to bottom of the scaffold, even though a constant layer thickness is used during the printing process. This change in pore size is due to the weight of the following layers. [30]. Scaffolds with more precise geometries were made using smaller layer thicknesses. A recent study showed that increasing the layer thickness by 30 μm not only improved production speed, but had no effect on the performance, dimensions and geometry of the spacesuits [31,32].

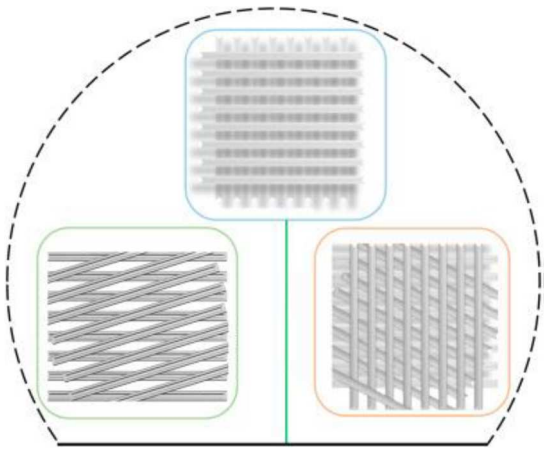

Figure 8 Schematic representation of scaffolds with different fiber orientations (90 °, 45 °, 60 °) [32]

A complex view of the structure created by additive manufacturing is their specific shape. There are studies that have looked at the effect of the shape of a spacesuit on its subsequent use. For example, the work of Zhang et al. focused on the printing of the so-called "Honeycomb patern" (see Figure 9). The shape of beeswax was chosen in the press to create spheroids, which made it easier to attach mesenchymal cells to human bone and enhance cell differentiation [33].

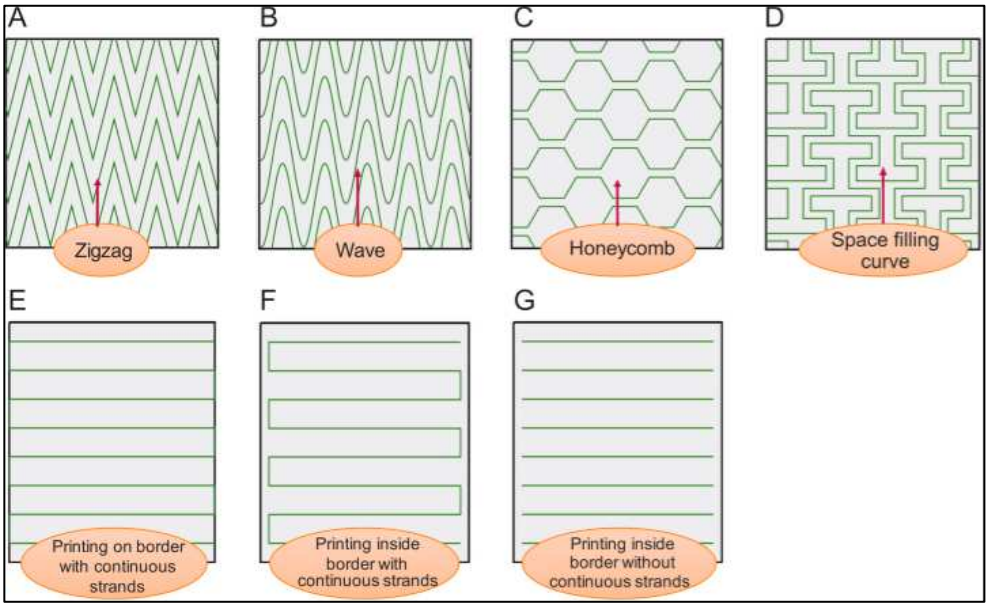

Figure 9 Different patterns for scaffold printing [32]

Several patterns are used to create hydrogel scaffolds, the choice of which depends on the required function of the scaffold. The zigzag pattern of hydrogel material, which due to its curvature requires support in 3D printing, was implemented to create a fabric structure. Square wave patterns are used to form a scaffold due to their ability to increase the diffusion of nutrients and drugs into the core of the fibers pressed layer by layer. Another type of pattern that is used for continuous extrusion to improve the printability of complex structures is space filling. This pattern has also been used to facilitate cell adhesion and grow faster [34,35].

3.3.2 Ink

Hydrogels from both natural and synthetic polymers are commonly used bioapplication materials because they can provide a highly hydrated environment suitable for cell adhesion along with mechanical integrity. Polycaprolactone (PCL), polylactic acid (PLA) and poly (lactic-co-glycolic acid) (PLGA) are synthetic polymers often used in 3D printing that exhibit high printing and mechanical stiffness. The lack of sites for cell adhesion, the high printing temperature, and the organic solvent needed to produce ink with these materials limit the production of cell contact structures, and thus for tissue engineering and drug applications [30,36,37].

Natural materials such as alginate, collagen, gelatine and chitosan are more gentle and less toxic to cells; however, they usually lack sufficient mechanical rigidity and longevity [38].

3.3.2.1 Hybrid inks

A preferred strategy for producing printing inks is to mix different types of materials with alginate. The aim is to create hybrid or composite scaffolds that show improved printing, mechanical properties and biological characteristics of alginate scaffolds. Pure alginate does not have the adhesion sites needed for cell attachment, and therefore scaffolds made from alginate and gelatine may be a solution for improving the biological properties of alginate scaffolds [39,40].

Gelatine is widely used to improve the mechanical properties and printability of hydrogel scaffolds. Mr. et al. analyzed the properties of the scaffolds containing gelatine together with alginate and found a high water retention rate. These studies suggest that the combination of different ink-producing materials can aid in printing and allow better control of the properties of the scaffolds to achieve the required scaffold functions [41].

3.3.2.2 Ink surface tension

Since the attraction of liquid molecules is stronger than the attraction of molecules in air, a liquid-air surface tension arises at this interface. The contact angle between these media indicates compressibility, which is referred to as interfacial energy.

A surface tension arises at the liquid-air interface because the attraction of the liquid molecules is more pronounced than the attraction of the molecules in the air. Surface tension is a liquid-air interfacial energy and can affect compressibility due to the contact angle between the two media. This angle indicates the degree of hydrophilicity of the scaffold, that is, the hydrophilicity of the scaffold can potentially support cell growth more because higher wettability improves biological behaviour [28].

In general, if the print substrate has a high surface tension compared to the surface tension of the ink, the ink will melt. Conversely, a low-energy hydrophobic substrate results in less spillage and a higher contact angle [28].

Most inks used in extrusion-based bioprinting have shear thinning effects, i.e., fibers applied at high flow rates have lower viscosities. Such fibers generally have smooth surfaces at the exit of the nozzle, leading to greater surface tension (see Figure 10). It follows that the high ink extrusion rate affects the lower viscosity of the extruded fibers [28].

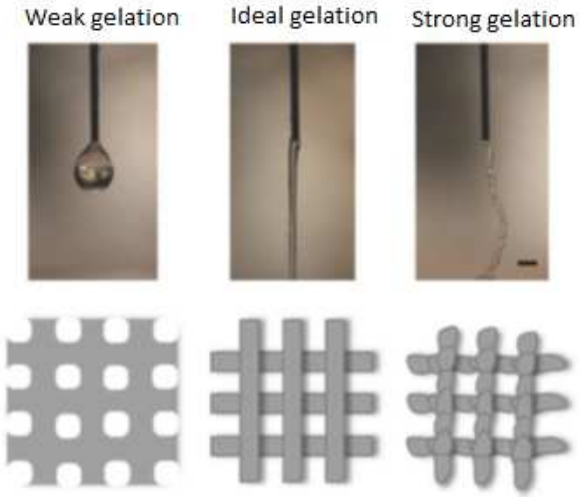

Figure 10 Influence of the degree of gelation on the quality of the final product

By affecting the surface tension of the ink and the substrate, printability can be improved or excessive ink expansion can be reduced. There are three approaches that can be used to manipulate material properties:

1. In order to avoid spillage of inks with a lower surface tension and at the same time to obtain a low energy surface such a printing substrate could be treated with a hydrophobic material. Substrate coating has been introduced as a suitable technique for modulating the surface energy of a substrate without directly affecting the ink material.

2. High viscosity ink can be used to achieve minimal ink spreading and spilling on the substrate. However, not all high viscosity inks are suggested due to the need for high extrusion pressure, as nozzle clogging may occur. Another consequence of the use of highly viscous material is unfavourable conditions for adhesion and subsequent cell viability.

3. Another approach to solving the problem of low print quality is to increase the speed of the process of crosslinking the hydrogel material or solidifying the extruded scaffold. This can be achieved by modifying the crosslinking mechanism (see Chapter 2.1) or the porosity of the scaffold - the higher the porosity, the faster the crosslinking rate [28].

3.3.3 The printing process

Printing parameters and the conditions under which printing takes place are especially important when printing complex 3D structures with different levels of porosity. Factors to consider in a printability study are the type of needle used for printing, the pressure developed, and the extrusion rate. Due to the interaction between the ink and the needle, the surface energy of the needle should also be considered [41].

For hydrogels, cross-linking during printing is a specific criterion, as pre-printing cross-linking increases the possibility of nozzle clogging, while cross-linking after 3D printing increases the likelihood that instability or disintegration of large structures will occur in large multilayer structures [28].

Thanks to their hydrophilic properties, biocompatibility and flexibility, hydrogels are used in several biomedical applications, such as drug carriers, absorbable sutures and injectable biomaterials. Protein-based polymers simplify some cellular functions, but the disadvantage is a relatively slow cross-linking rate compared to polysaccharides such as chitosan, which have polymerization rates more suitable for their printing [28].

Physical hydrogels are formed by a variety of reversible bonds, including hydrogen bonds, hydrophobic interactions, electrostatic / ionic interactions, as well as combinations thereof. The sol-gel transition in physical gels is usually triggered by stimuli such as pH, temperature, magnetic field or the addition of suitable ions. Physical chitosan hydrogels do not need polymerization aids during crosslinking, which makes them suitable for the cells due to their low toxicity. However, these hydrogels often have low mechanical stiffness and it is also difficult to reproduce their properties, such as pore size or dissolution rate [28].

I. Laser-based systems based on the photopolymerization process.

II. Systems based on extrusion of (pre) polymers by means of a nozzle.

III. Printer and binder injection based systems [25].

4 Results and discussion

While AM technologies used in the processing of metals, ceramics and thermoplastic polymers have inspired the field of biofabrication, these "classic" AM approaches generally involve the use of organic solvents, high temperatures or crosslinking agents that are not compatible

with living cells or biomolecules. Hydrogels must be processed under more favourable cell conditions, which was taken into account during the development of the methodological manual for the production of hydrogel scaffolds by AM technologies.

Another criterion is to solve the problem with support structures during printing. Although the need for support materials is common in many AM techniques, for soft hydrogels with a modulus of elasticity <100 kPa, this problem has been solved by using the FRESH method, which uses a support bath. The liquid hydrogel is then pushed into it and the support bath thus holds the hydrogel in the desired place thanks to buoyancy until it solidifies.

5 Conclusion

Increasing demands on medicine itself force rapid progress in the field of materials applicable in this area. the conditions for approval of materials are very high. Today, only the biocompatibility of the material is not enough, but the material must meet a number of requirements and physical advantages. In the field of 3D printing, the use of hydrogel materials, which have high potential in the field of tissue engineering and regenerative medicine, is important. The printing process itself is a complicated event and several requirements must be met.

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