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Bio–inks for 3D extrusion–based bio–printed scaffolds: Printability assessment

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Abstract: 3D bio–printer is a new technology that requires to be integrated into several areas, including medical technology. However, before design and apply at large scale it is required to establish several biophysical parameters and particularly printability. In the present work, general characteristics of extrusion method, bio–inks and scaffolds are reviewed. Printability analysis on 3D bio–printing are also included.
Keywords: 3D Bio–printing; Bio–inks; Printability; Hydrogels.

Introduction

3D–bioprinting (3DB) is a multilayer–based approach run on computer program designs and used to produce complex devices for several purposes in many areas such as biomedicine, biotechnology, among others.[1] The integration of technologies from bioengineering, materials science, cell biology, physical chemistry and medicine to the bio–print field ensures a promising future for this innovative technology[2]. The most current approach of 3DB is the potential application in biomedical engineering and translational medicine, which consists on the development of customized scaffolds of 3D porous structures with interconnected channels made of bio–inks based on biomaterials with active biomolecules containing or not cells[3,4]. The main application of 3D scaffolds is the production of biocompatible constructs for tissue/organ regeneration.[5] In addition; scaffolding bio–print can be applied for the development of high–throughput assays, drug discovery systems, and others[5].

The main bio–ink properties can be grouped in biological, chemical and biophysical characteristics (Figure 1).

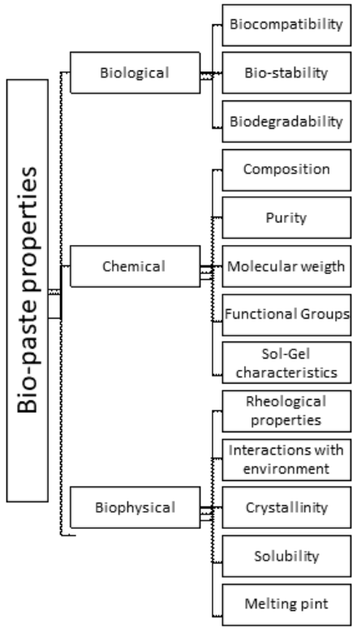

Figure 1 – Main characteristics of bioinks classified in groups of properties.

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Extrusion method, bio–inks and scaffolds general characteristics

Extrusion method is the most studied additive manufacturing technology; this is related to advantages such as precise deposition, cost–effectiveness, simplicity, process speed, homogeneous distribution of bioactive components, tailoring, versatility and predictability[6]. In this method, bio–inks are extruded through a print head using syringes with tips or specific nozzles by either pneumatic pressure or mechanical force but without heat requirements. Additive manufacturing technology has demonstrated its great potential in producing functional scaffolds for biomedical applications. All molecules used for the development of scaffolds must possess biocompatibility, no toxicity and proper biophysical properties to induce molecular bio–recognition and ensure an optimal environment for molecules and/or cells[7].

In the extrusion methods, one of the most important factors for 3DB procedure is the rheological behavior of bio–inks. During the extrusion, the material flow from the nozzle and the fusion of layers results in scaffolds with controlled pore size, morphology, and interconnectivity[1]. Apparent viscosity, defined as the ratio of shear stress to shear rate is a relevant parameter, since should be low enough to allow the extrusion process of clear filament and mechanically strong to support the deposition of upper layers without compression and/or changing the matrix shape[4,8]. Some research groups have demonstrated the advantages of rheological characterization to systematize 3D printing methodologies and to develop mathematical models that help to gain a deep understanding of non–Newtonian fluid models[8–10].

In order to facilitate tissue regeneration, scaffolds must be designed to provide a proper environment for cell growth, which generally depends on both, selection of materials but also geometrical features such as internal structures and pore size distribution. Another critical issue are the mechanical properties of the scaffold that must match those of the original tissue to be used and/or repaired[11]. Moreover, scaffold mechanical properties such as stability and degradation kinetics must be adapted to the specific tissue application and requirements in order to guarantee the proper mechanical functions and to accomplish the rate of the new–tissue regeneration and/or formation[8].

Printability analysis on 3D bioprinting

Recently, bio–ink research efforts have been made to develop new materials with the aims of improving biocompatibility and biofunctionality[4]. Based on the fast increase in the knowledge associated with biomaterials, cell–scaffold interactions and the ability to bio–functionalize/decorate bio–inks with cell recognition motifs (e.g. biomarkers, mucoadhesive molecules, etc.), it is also important to consider the "printability" of these novel materials[12].

Extrudability is defined as the ability to eject a paste through a nozzle without considerable cross–sectional deformation and acceptable degree of splitting/tearing of the resulting filament[13]. In fact, the extruded filament width is expected to be similar to the nozzle diameter in order to obtain a good shape fidelity and correlated to the computer aided design (CAD) model since the nozzle diameter value is included at initial set parameters of the equipment.

In previous work, seven possible filament types produced by extrusion printing of alginate–gelatin blends by varying the materials properties and operating conditions were reported. The work used continuous and defined filaments that show swelling, equivalent or stretched diameter regarding to nozzle were considered well–made filaments and they are favored for 3D printing due to the defined geometries results. Irregular filaments with rough surface, over–deposition material, compressed material or discontinuity and should be avoided due to the uncontrollability of the morphology and/or diameter of such filaments[14].

There are different reasons why irregular filaments are obtained, the most frequent are low pressure of extrusion motor, nozzle obstruction due to large particles or bubbles formation in the mixture, high paste viscosity, incomplete mix of components, and excessive shear forces inside the nozzle during printing and "pinch" of the filament due to low paste viscosity[15–18].

The printed filament must show clear morphology with smooth surface and constant three–dimensional widths[13]. The good bio–ink printability result into regular grids and square holes displayed in linear scaffolds constructs, on the contrary, the upper layer could fuse within the lower layer creating approximately circular holes if the extruded filament showed a more sol–like state with low–viscosity[4].

The dimensionless parameter used to characterize the extruded filament is circularity (C) of an enclosed area that can be determined by the follow equation:[4]

$$C = \frac{4\pi A}{L^2} \qquad [1]$$

where L is the filament perimeter and A is the cross–section area.[4]

Circular sections have the highest circularity (C= 1). The closer the C value is to 1, the closer the shape is to a circle.

For a square shape, circularity is equal to π/4. The bio–ink printability (Pr) dimensionless parameter is based on square shape and defined using the following equation:

$$\mathrm{Pr} = \frac{L^2}{16\,A} \qquad [2]$$

where L is the filament perimeter and A is the cross–section area.

For an ideal printability status, the interconnected linear channels of scaffolds display square shape with Pr value of 1[4].

Three typical pore shapes are displayed in **Figure 2**. Scaffolds A, B and C were developed using the cartesian 3D bio–printer "NBM–FAB––CINDEFI" commanded by Arduino open hardware and Marlin firmware designed and constructed in the NBM Laboratory. The equipment was designed to extrude viscous materials using a syringe with a nozzle size

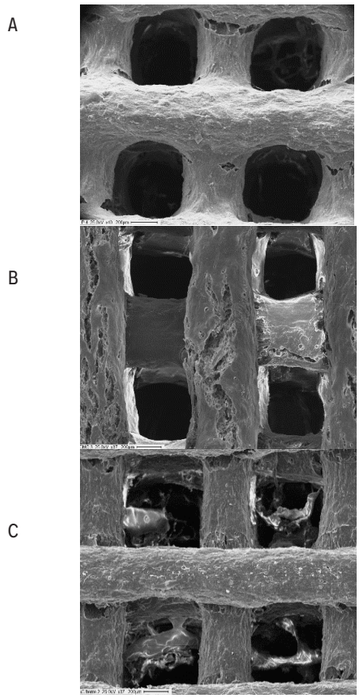

Figure 2 – SEM images and printability of pectin scaffolds under three typical pore shapes. A, pore shape closer to a circle made of pectin; B, little distorted square pore shape made of pectin and carboxymethyl cellulose and C, pore shape closer to a square made of pectin and microcrystalline cellulose.

of 0.2 mm or 0.4 mm. Scaffold A was printed with pectin bio–ink, B with pectin plus carboxymethyl cellulose and C with pectin plus microcrystalline cellulose. Pr values were analyzed by Image–J software using Scanning Electron Microscopy (SEM) images of printed scaffolds to establish the perimeter and the area of interconnected channels (n= 10). The scaffold C presents the best square interconnected channels, being it semi quantitative printability value the closest to 1 with the lowest SD. High Pr values are proportional to high bio–ink viscosity, which reveals the relevance of hydrogel rheological properties and connected with the physicochemical composition of the bio–ink paste. These parameters play an important role in controlling the resolution and shape fidelity of the 3D bio–printed structures.

Conclusions

The convergence of engineering techniques and life sciences evolved to develop the extrusion–based 3D bio–printing from a simple technique to one able to create diverse scaffolds from a wide range of biomaterials, bioactive molecules and cells types. The development and formulation of extrudable bio–inks has been a major challenge in the field of biofabrication. Bio–inks must not only display adequate rheological and mechanical properties for the chosen application but also to show high biocompatibility as well as bio–printability. Biological, and physicochemical requirements are quite studied in material science field while extrudability and printability assessing of bio–inks still needs to be carefully examined to enable robotic bio–printing. The present review summarizes printability concepts and displays some approaches to it analysis. For future, it is expected mathematical models, rheological assays, qualitative and quantitative physical analysis on 3D bioprinting applications to be standardized.

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