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Applicant: Sofia Moroni

THE GROUNDBREACKING 3D PRINTING TECHNOLOGY IN THE PHARMACEUTICAL FIELD

Introduction

Additive manufacturing or three-dimensional printing (3DP) was patented in 1986; however, it became popular only during the last decade thanks to a higher accessibility and lower costs of this technology. It consists of fabricating solid complex objects starting from a virtual Computer Aided Design (CAD) model through the consecutive layer-by-layer process. Owing to the several advantages provided, this technique is revolutionizing the traditional fabrication methods in numerous fields, including the pharmaceutical world. Indeed, it allows to fabricate complex designs in an accurate, reproducible, cost-effective and timely manner that can't be achieved through conventional techniques.[1]-[2]-[3]Additive manufacturing comprises various methods such as binder jetting, material extrusion and stereolithography; Table 1 compares the material, resolution, biomedical applications, advantages and drawbacks of the primary techniques.[4]

Printing Technique	Material	Resolution	Biomedical Applications	Advantage	Disadvantage
Binder Jetting	Sand Metal powder	50–400 µm	Degradable (Fe-based alloys) metallic implants [31]. Generally used for hard, mineralized tissues	Low cost, fast, color printing, no support structure needed, large objects	Low strength, requires post-processing, powders pose a respiratory hazard
Directed Energy Deposition	Metal Nylon	250–500 µm	Limited use in medical applications	Fast, composite materials, can patch defects on existing objects	Expensive, slow, low resolution, requires post-process machining
Material Extrusion (FDM[1])	Hydrogels Thermoplastics Ceramics Bioinks	100–200 µm	Bioprinting of scaffolds for cell culture, tissue and organ development (soft tissues) [32] Production of rigid and soft anatomical models for surgical planning	Color, low cost, accessible, composite materials, open source designs	Slow, anisotropy, lower resolution, nozzles impart high shear forces on cells
Material Jetting/Inkjet (MJ[2], DOD[3])	Photopolymer Bioinks	20–100 µm	Bioprinting of scaffolds for cell culture, tissue and organ development (soft tissues) [33]	Good resolution and cell viability	Slow, material waste
Powder Bed Fusion (SLS[4], DMLS[5]/SLM[6], EBM[7])	Thermoplastics Metal Powder Ceramics	100–200 µm	Metallic implants; dental, craniofacial and orthopedic [34] Temporary and degradable rigid implants [35]	Strong, fast, no solvents required	Most expensive, medium resolution, post-processing required
Sheet Lamination	Paper Ceramics Metal	~1 mm	Macroscopic anatomical models	Low cost, composite materials, no support structure needed	Slow, lots of material wasted, delamination
Stereolithography (SLA[8], DLP[9])	Photopolymer Bio-resin	1.2–200 µm	Bioprinting of scaffolds for cell culture, tissue and organ development, can be used for both soft and hard tissues [36]	High resolution, fast, very good cell viability, nozzle free	Raw material toxicity, limited material selection, possible harm to DNA by UV
Spheroid assembly	Bioink Organoids	100–200 µm	Tissue and organ development, soft tissues [37]	Biologically active models, scaffold free, freeform fabrication	Fragile raw material, requires subsequent spheroid fusion

Table 1: comparison of the principal techniques employed in 3DP. [4]

Nowadays, the 3DP has found wide applications also in the pharmaceutical field, for instance in drug delivery and tissue engineering.[4] The interest in the 3DP for the production of pharmaceutical forms is due to the high versatility and adaptability of the method that allow the complete personalization of the product fabricated for the patient. Indeed, by modifying the design and the amount of loaded drug it is possible to change the release kinetics and dosage profile in order to achieve for example the minimization of side effects and the increase of patient compliance. [2-5]

In this context, several implantable delivery devices are currently in commerce, for instance: Nexplanon®, since 2010, it is well established as a long-acting contraceptive[6]; Vantas® is once-yearly administered for the treatment of prostate cancer[7]. More recently, researchers are focused on the development of innovative devices such as microneedles that represent one of the most attractive and promising products.

Microneedles are a versatile platform, due to their ability to deliver a wide range of molecules, such as insulin, vitamins and antibiotics. They can be fabricated with several materials (e.g. silicon, glass, metals, polymers); in addition, they can assume various design, for instance, they can be coated, hallow, solid or dissolving. Moreover, they can target different tissues, for instance, they can be applied to the eye, the oral mucosa or the skin [8].3DP is a really attracting technique for the production of these systems due to the possibility to have a complete personalization of the device in term of size and shape.

Despite the significant advantages conferred, the application of 3DP in the pharmaceutical field requires further investigations, indeed, the primary issues are related to the lack of regulatory guidelines and the choice of the materials as few compounds possess the optimal characteristics to be printable, for instance in terms of thermal stability as high temperatures are required for the process.[3-5]

Aim of the project

The main goal of the project is to acquire further knowledge on 3DP.
More specifically the project aims to:

- Identify the best 3DP technique to prepare pharmaceutical forms;
- Investigate the formulation parameters to obtain optimal printable mixtures;
- Characterize the developed systems, on the basis of, morphology, surface chemistry, and stability;
- Evaluate the cytocompatibility *in vitro*;

- Assess the interactions with cells;
- Explore the *in vivo* behaviour.

Methodology and expected results

Product design and manufacture

The first step will concern the optimization of the production process, considering the starting materials and the final product desired, different manufacturing methods will be considered. The product will be designed using the CAD software and printed; in order to obtain an optimal device, several parameters will be evaluated such as the print speed, the temperature and the layer heigh.

Product Characterization

- ***Scanning Electron Microscopy:*** it can be employed to visualise the morphology of the device;
- ***FT-IR spectroscopy:*** it is used to chemically characterize the device, for instance, it can be applied to verify the interactions between the materials employed;
- ***Texture analysers:*** it allows to verify the mechanical properties of the product;
- ***Differential Scanning Calorimetry (DSC)*****:** the thermal behaviour of the printed device and the materials employed can be analysed using the DSC;***HPLC:*** it is useful to quantify drugs to perform the release studies.

Cytocompatibility in vitro and interactions with cells

- The ***biocompatibility*** of the systems can be assessed on different cell lines representative for the desired target, for instance endothelial cells, cancer cells or myoblasts. The aim of the test is to evaluate the possible toxicity effect of the systems in cells;
- The ***bioactivity*** of the product can be performed by using different techniques to evaluate if the drug release is effectively enough to treat the selected disease. An example is the measurement of microbicide effectiveness against a specific pathogen such for instance *Candida albicans*.

In vivo studies on diseased models

Overall, the results expected at the end of the project consist of the successful development of innovative systems that prove to be stable, biocompatible both *in vitro* and *in vivo* and that demonstrate a potential role to improve the state of care of injured patients.

References

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Feasibility of the three-years project:

Time Period	***Activities***
First Year Nov. 2020 – Oct. 2021	• Acquisition of knowledge about ▪ Three-dimensional printing ▪ Applications ▪ Materials • Development of protocols and devices for the preparation of innovative systems • Optimization and characterization of the developed systems • Visiting period abroad

Second Year Nov. 2021 – Oct. 2022	• Further acquisition of knowledge about the target and the delivery systems produced • Visiting period abroad
Third Year Nov. 2022 – Oct. 2023	• Final *in vivo* and *in vitro* studies • Final thesis