FUNCTIONALLY GRADED ADDITIVE MANUFACTURING TO ACHIEVE FUNCTIONALITY SPECIFICATIONS OF OSTEOCHONDRAL SCAFFOLDS

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease, characterised by cartilage loss and changes in bone at the interface of a joint resulting in pain, stiffness and reduced mobility. OA is one of the most prevalent chronic conditions as identified in Bone and Joint Decade. According to the World Health Organisation 40% of people over the age of 70 have OA. This joint disease affects around 0.4 billion people with patients in Europe accounting for up to 30%. The figure is set to increase with the ageing problem. Patients with OA often suffer pain and loss of mobility and go on to require an end stage total joint replacement. This would happen when the loss of quality of cartilage and bone at the joint interface has significantly reduced the quality of life of the patient, and non-surgical treatments are no longer effective.

Current non-surgical treatments for OA involve non-steroidal anti-inflammatory drug administration. Surgical treatments include osteotomy, abrasion arthroplasty, microfracture and autologous chondrocyte implantation (ACI). This is a two stage surgical procedure with the associate costs and infection being the main concern. For small osteochondral defects, microfracture (MF) marrow stimulation and for large cartilaginous defects the autologous chondrocyte implantation are considered as necessary treatments. However, MF produces fibrocartilage not native hyaline cartilage. While for defects that have progressed to a stage that affects the subchondral bone, other treatments are no longer effective and joint replacement operation is the only alternative.

The demand for innovative therapeutic alternatives for complete healing of OA is significant. The treatment of cartilage and osteochondral (OC) defects remains a challenge since treatments so far have failed to achieve complete restoration of the properties of joint cartilage. Many new technologies, such as osteochondral tissue engineering, have been studied and applied to repair osteochondral defects. Commercially available osteochondral scaffolds have been used in patients with OC defects. However, no products have so far demonstrated to provide biomechanical properties suitable to promote the durable regeneration of large OC defects [1]. The main issue with these commercially available OC scaffolds is poor cartilage fill associated with fibrocartilage formation.

The aim of this paper is to define the functionality and performance which would be required for intended clinical applications in the treatment of osteoarthritis. Also, to show that the capabilities of 3D bioprinting and functionally graded additive manufacturing scaffolds are suitable to meet most of these requirements.

COMMERCIAL OSTEOCHONDRAL SCAFFOLDS AND CLINICAL PERFORMANCE

Treatments using tissue engineering methods have been established and are promising for the treatment of small osteochondral defects.

Biphasic scaffolds have represented many relevant progresses for osteochondral reconstruction in vivo or preclinical test. Multi-layered scaffolds, consisting of bone- and cartilage-like layers, seem to be the most promising approach to achieve the regeneration of OC defects [2]. Moreover, a few novel bilayer scaffolds have been approved for clinical implementation: MaioRegen® [3-4] and TruFitTM Plug [5-6].

MaioRegen® is a bilayer scaffold mimicking the OC unit. The superficial layer is composed of Col I and is similar to the cartilaginous tissue, while, the bottom layer consists mostly of magnesium-enriched hydroxyapatite (Mg-HA), similar to the subchondral bone structure [7]. The intermediate layer of collagen and Mg-HA replicates the tide-mark. TruFitTM plug is a bilayer scaffold composed of PLGA fibre and calcium sulfate ($CaSO_4$).

MaioRegen® has been systematically studied in patients. The international knee documentation committee (IKDC) reported that subjective score of the suffer knee was significantly improved. Similar positive conclusion was confirmed by the visual analogue scale and Tegner scores at 24 months after implantation [8]. These results showed that this was a good strategy for OCD treatment but abnormal magnetic resonance imaging findings were presented [9]. Another study was carried out in 11 patients for the treatment of tibial plateau lesions. An acceptable clinical behaviour was reported at 2 years follow-up [10]. Recently, Christensen et al. [11], investigated the analogous results of bilayer MaioRegen® for osteochondral defect repair after 1–3 years clinical and radiological observation. Incomplete cartilage repair and poor subchondral bone repair was found at 1 and 2.5 years follow-up. Nevertheless, the clinical scores were significantly improved.

Agili-C™ (CartiHeal, Israel) is another recently developed osteochondral scaffold consisting of a natural crystalline aragonite, derived from corals, to which hyaluronic acid (HA) is added [12]. The natural aragonite, possess a nano-rough structure as well as interconnecting porosity that allows to stimulate cell adhesion and proliferation as well as matrix production.

To summarize, for the success of osteochondral tissue engineering the primary requirements of scaffolding materials include biocompatibility, biodegradability, mechanical stability, and pore structure:

Biocompatible and degradable materials

It is well known that scaffolds must be fabricated from biocompatible materials which do not stimulate immune responses or foreign body reactions. In addition, the biodegradation of scaffolds during in vivo treatment should closely match tissue growth rates [13]. Facilitating regeneration of cartilage requires that the implanted scaffold remain stable for at least two-three weeks. Stability of the scaffold in this period allows sufficient time for the composition of support structures for subsequent regeneration of tissues.

Mechanical stability

Osteochondral interfacial tissue has different mechanical strengths depending on the property at each stratified layer. Mismatched viscoelastic properties of osteochondral tissue lead to stress disparities between cartilage tissues. Superficial cartilage can withstand a local compressive stress of 0.08– 2 MPa, tensile modulus of 5–25 MPa, and equilibrium shear modulus of 0.05–0.25 MPa [14]. These differences arise from the biological and chemical composition and thereby from mechanical strengths in each zone. In order to optimize resistance in osteochondral tissue, superficial collagen exists parallel to the shear direction, while collagen in the deep zone is perpendicular to the surface. Owing to this highly organized structure and its properties, artificial recreation of this tissue is still challenging. Intensive progress on remodelling cartilage has been made using transforming growth factor-β1 (TGF-β1) and mechanical stimulation to improve its tensile modulus up to more than 3.4 MPa [15].

Pore structure

The pore structures affect the cell responses and their further organization in the tissue, regulating cell invasion, vascularization, and tissue regeneration in most scaffolds. In several studies on the effects of pore size, scaffold structures composed of porosity higher than 50% and pores larger than 300 µm is recommended to achieve direct osteogenesis with enhanced vascularization [16]. On the contrary, pores of 90–120 µm have been suggested for favourable chondrogenesis, where MSCs proliferate and form cartilage tissue on the scaffold [17].

HOW ADDITIVE MANUFACTURING CAN COMPLY WITH REQUIREMENTS FOR THE CLINICAL PERFORMANCE OF OC SCAFFOLDS

The Additive Manufacturing (AM) technology is based on solid freeform manufacturing for the direct production of complex parts without resorting to specific moulds and tools [18]. According to ISO/ASTM 52900:2015 [19], AM is defined as “process of joining materials to make parts from 3D model data, usually layer upon layer, as opposed to subtractive manufacturing and formative manufacturing methodologies”. AM is a multidisciplinary field requiring close interaction between design, material, technology and information and communication technologies (ICT). At present, AM involves procedures for the addition of material layer by layer starting from 3D solid CAD. ISO/ASTM 52900:2015 has categorized AM processes into seven areas:

1. Binder jetting: It uses liquid bonding agent to join powder materials.

2. Directed energy deposition: Focused thermal energy is used to fuse materials by melting as they are being deposited.

3. Material extrusion: The material is selectively dispensed through a nozzle.

4. Material jetting: Droplets of build material are selectively deposited.

5. Powder bed fusion: Thermal energy selectively fuses regions of a powder bed.

6. Sheet lamination: Sheets of material are bonded.

7. Vat photopolymerization: A liquid photopolymer in a vat is selectively cured by light

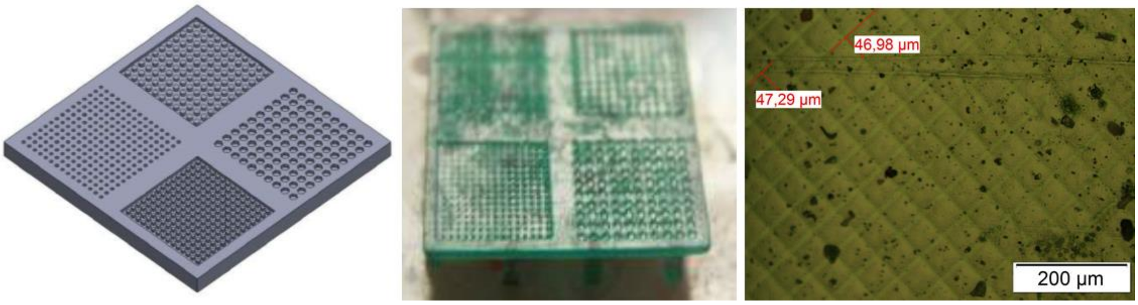


Figure 1. Micro features by DLP part (total part size 7x7x0.5 mm). Pixelation structure due to resolution of DLP. Courtesy University of Las Palmas de Gran Canaria.

Some of these procedures are very suitable for micro-manufacturing, with high potential in tissue engineering due to the small size of features or texture. For example, in the group of Vat photopolymerization is well known as the Digital Light Processing (DLP), which is based on an array of micro-mirrors suitable to be oriented into two positions, moved by micro-actuators. The UV light is reflected by these micro-mirrors to the layer of liquid photopolymer, curing each layer like a unique mask [20]. The resolution of the cured mask is defined by the number of pixels. Figure 1 shows the level of detail for small features (diameter of pins 0.15 mm, side length of squares 0.09 mm, diameter of holes 0.1 mm). Also the pixilation due to the resolution of the mask (in this example the size of each pixel is about 40 microns) can be observed. The availability of hydrogels based on photopolymers becomes this DLP system in a very promising technology for bioprinting at micro level, not so easy to achieve in extrusion based technologies such as the bioplotters as described in the following paragraphs.

Among the previous methods, powder bed fusion is mainly used for titanium alloy scaffolds (SLM or EBM) [21]. However, the bioinert nature of this material inhibits osteointegration with the surrounding tissue. Several companies have developed bioprinters to take advantage of the capabilities of additive manufacturing to produce complex scaffolds. Several natural and synthetic polymers, bioactive inorganic materials, and their combinations have been employed for bone and cartilage tissue engineering and regeneration. Nevertheless, not all the biomaterials are suitable to be printed by these technologies. In particular for those extrusion based bioplotters the extrudability of either biopolymers or bioinks is the main factor to take into account from the point of view of the processability. Temperature and viscosity are key factors in order to achieve good quality of material as well as accuracy of the porous/lattice structure. Many natural biopolymers (chitosan, alginate, collagen) processed as hydrogels, have the advantage of less restrictive parameters of processing and no needs for toxic solvents. These can be either processed by the method of material extrusion (with chemical crosslinking) or Vat photopolymerization (crosslinking by UV light) .Otherwise, material jetting requires very low viscosity of the biopolymer (below 10 cP) [22] limiting the availability of materials. Table 1 shows a summary of some commercial extrusion based bioplotters as well as available bioinks [23].

Table 1. Commercial bioplotters and biomaterials for 3D printing

Model, Company	Material
3D Bioplotter, Envisiontec	Ceramic/metal pastes (HA, TCP, Ti), thermoplastics (PCL, PLLA,PLGA), Hydrogels (Agar, Gelatin, Soy, Hyaluronic acid, Alginate, fibrin, chitosan, collagen), acrylates (UV curing)
BIO X., CELLINK	Alginate/nanocellulose, collagen, gelatin methacrylate, Pluronic

	F127 (polypropylene glycol/polyethylene glycol), TCP, PCL,
Biofactory, RegenHU	PEG, gelatin, hyaluronic acid-based, Calcium phosphate
Allevi 6, Allevi	PLGA, PCL, Pluronic 127, gelatin methacrylate, collagen, alginate, fibrin, Polyethylene glycol diacrylate

One relevant feature of a bioplotter is the option of depositing multi-materials by multi print heads. This option provides making multimaterial scaffolds combining, for example, hydrogels with biopolymers, following different strategies. For instance, Shim, et al. [24], combined synthetic materials such as PCL or PLGA with hyaluronic acid, gelatin or collagen based, by alternative extrusion of these materials in a multi print head extruder, observing a significant improvement of mechanical properties.

Integration of chondral phase and osseous part via stereolithography was carried out by anchoring a cured PEG hydrogel tightly to the underlying ceramic substrate [25]. Other works have investigated innovative bilayered porous silk fibroin-based scaffolds (Figure 2), developed by combining a horseradish peroxidase (HRP)-crosslinked silk fibroin (SF) layer with a HRP-crosslinked SF/β-Tricalcium phosphate layer [26].

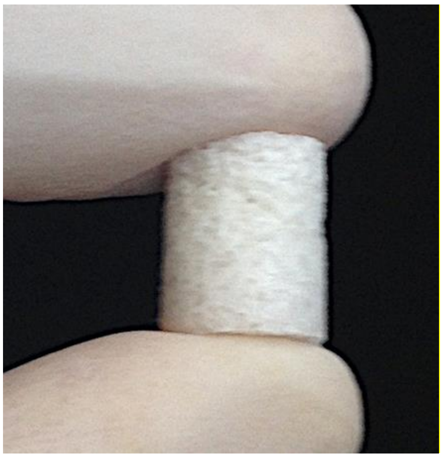


Figure 2 Bilayered porous silk fibroin-based scaffolds, combining a horseradish peroxidase (HRP)-crosslinked silk fibroin (SF) layer with a HRP-crosslinked SF/β-Tricalcium phosphate layer. Courtesy University of Minho.

Liu C. and Blunn G.[27] have patented a biomimetic OC scaffold, by a gradient structure formed by titanium matrix, PLA junction and PLGA infiltrated collagen layer (Figure 3).

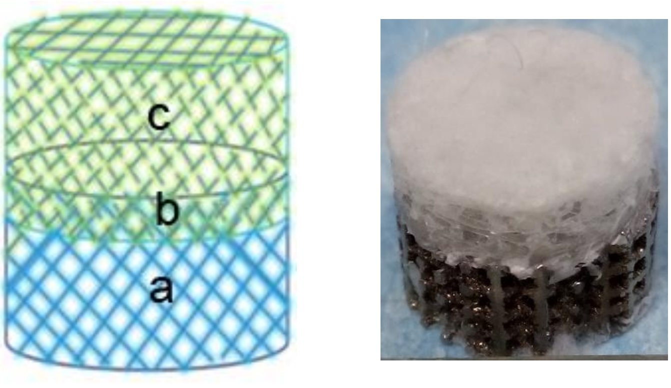


Figure 3. Gradient structure. a: Titanium matrix b: PLA junction c: PLGA infiltrated collagen layer. Courtesy University College London.

Another promising procedure is the opportunity of **Functionally Graded Materials (FGM)**, which in the context of AM is named **FGAM (functionally graded additive manufacturing)**

FGAM is a single additive manufacturing by gradationally mixing materials or modifying process parameters to fabricate freeform geometries with variable-property within one component. Functionally graded materials can be classified as follows (Figure 4):

- **Chemical composition FGM -** With two options: a) single phase as a result of the solubility of the chemical elements of one phase in the other phase. Varying chemical composition because of the solubility; b) Multiphase chemical composition. The phases and chemical composition are made to vary across the bulk volume of the material.
- **Porosity /cellular gradient FGM-** a) Porosity/cellular density gradation: The porosity density is produced with the density of porosity changing with respect to the spatial position across the volume of the material; b) Pore/cell size/shape gradation: Varying the pore/cell sizes or the pore shape, or both.
- **Microstructure gradient FGM-** The graded microstructure would result in a gradual change of the material properties with respect to position.

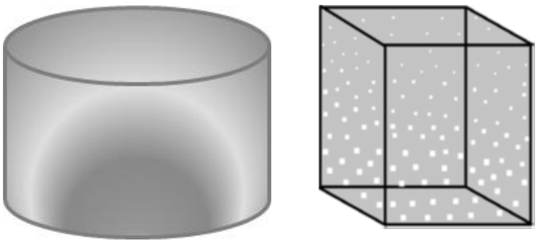
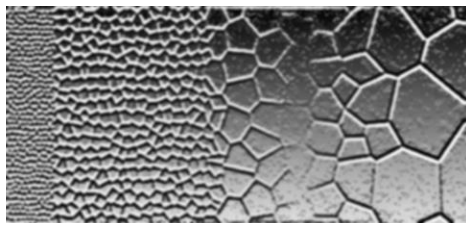


Figure 4. FGM. a. Chemical composition FGM. b. Porosity FGM. c. Microstructure FGM



Many applications of FGAM have been carried out in the medical field so far [28]. Chua et al. [29], and Sudarmadji et al. [30], presented a methodology for designing functionally graded tissue engineering scaffolds. It was composed of a library of 13 polyhedral geometric models which were obtained using Boolean operations to design an optimum scaffold. Yoo et al. [31], presented another general design framework for 3D internal scaffold architectures to match desired mechanical properties and porosity simultaneously.

In order to facilitate the manufacturing process, of OC scaffolds with FGM property, in a bioplotter the following capabilities should be implemented:

- Capabilities for multi-material and modification of composition of each material (for example with multi-feeder and mix chamber).
- CAD software with option of defining graded material in the 3D solid file.
- CAE software (FEM analysis) suitable to calculate with graded material.
- Exchange file from CAD to AM system with the option of FGAM. The well-known STL file does not implement this option.

There is a lack of CAD/CAE systems with capabilities of FGM , so it limits the automatic process from CAD to 3D printed FGAM, requiring additional tools for achieving a real FGAM. Nevertheless, different initiatives are taking place such as the exchange file formats such as

AMF and FAV. These new formats will necessarily be the replacement of STL in the future although there is still a slow process of implementation in the commercial CAD systems.

Additive Manufacturing Format (AMF) has been developed in the context of an ISO-ASTM standard, ISO/ASTM 52915:2016 [32]. This format is very suitable for multi-material specification, mixed graded materials, composites and porous materials. There are three options for enabling FGAM:

- Representation of FGAM by a mathematical function. A relevant utility is the element <composite> that is used to specify the ratio of the composition as a constant or an equation dependent on the x, y and z coordinates.
- Volume texturing, allowing to store the distribution in space of a certain material through a sequence of 2D textures, mapped in the volume of the object.
- AMF supports voxel representation, which is the three-dimensional equivalent of pixels. Common uses of voxels include volumetric imaging in medicine. The advantage of this is the option of assigning a particular material to one voxel.

Although many of the capabilities of AMF have been already included in many CAD software, unfortunately some of them, suitable for FGAM, are still under development.

Fabricatable voxel (FAV), created by Fuji Xerox [33] , expresses 3D data in the form of voxels arranged three-dimensionally. For each voxel, users can define various attribute values, including colour information and material information. Users can also control the relationships (e.g., connection strength) between different voxels. With this data format, it is possible to create designs with multiple materials.

PROPOSED METHODOLOGY TO FABRICATE FUNCTIONALLY GRADED OC SCAFFOLDS

Although there are some gaps to fully produce functionally graded OC scaffolds, due to limitations in the FGAM technology as discussed in the previous section, an ideal general methodology could be approached (Figure 5) to improve the existing procedures. In this methodology the 3D geometry could start from 3D CAD (as usual) or from 3D image coming from μCT with voxels as basic element, mimicking, for example, a Harvesian architecture [34]. Mechanical analysis by Finite Elements Method (FEM) allows to predict mechanical behaviour but this approach proposes also a **dynamic optimization**. The concept of this **dynamic optimization** is to consider the modification (depending on the time) of either geometry or physical properties of the material due to the degradation process of the biomaterial and the new cell tissue in the scaffold. In case of FGAM OC scaffold, this dynamic optimization enables optimal design and distribution of material but taking into account constraints regarding viability and efficiency of cell culture (for instance, level of porosity). Some authors have been working in optimization of lattice structures when geometry is changing with the time. For example, R. Paz et al. [35], optimized a 4D printing geometry, of shape memory part, for two different scenarios when an external stimuli deforms the part, based on genetic algorithm as method for optimization. Once the dynamic optimal solution is achieved, the 3D geometry is exported either to AMF or FAV and the slicer interface imports such an exchange format to define the routes, processing parameters and print heads, combining the graded materials to make the graded OC scaffold in a multi-head bioplotter .Note that a specific strategy is needed when combining hydrogels or biopolymers melting at very low temperature with those biopolymers at high temperature (for example programming some delays to cold the deposited biomaterial).

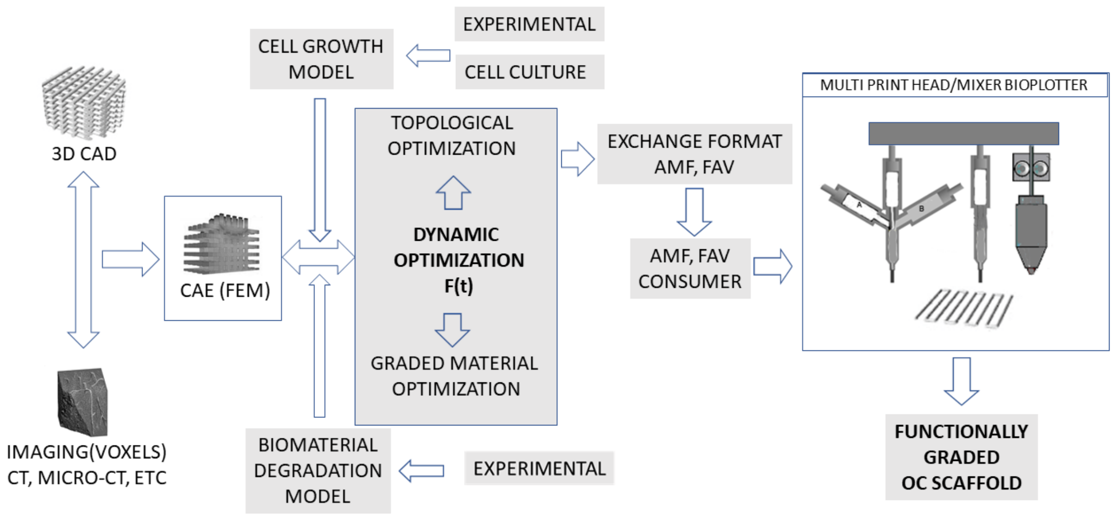


Figure 5. General methodology for design and manufacturing Functionally Graded OC scaffolds

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