Review on applications of Rapid Prototyping in Tissue Engineering and Medicine

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Abstract — Rapid prototyping is a group of techniques used to quickly fabricate a scale model of a physical part or assembly using three-dimensional computer aided design (CAD) data. Construction of the part or assembly is usually done using 3D printing or "additive manufacturing" technology. The most interesting and challenging applications of Rapid Prototyping technologies are in the field of medicine. RP medical models have found application for planning treatment for complex surgery procedures, training, surgical simulation, diagnosis, design and manufacturing of implants as well as medical tools. One such area where the application of Rapid Prototyping has been rapidly developing is the field of tissue engineering and guided tissue repair. Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physio-chemical factors to improve or replace biological functions.

In this paper, we present a comprehensive review of the applications of Rapid Prototyping in tissue engineering such as biodegradable implants, their manufacturing processes such as 3D dispensing RP process, biomaterials like polymers, hydrogels and composites needed to make these implants and the versatile application potential of new hydrogel scaffolds. Also in contrast to conventional RP systems, which mainly focus on melt processing, the 3D dispensing RP process (3D plotting) which can apply a much larger variety of synthetic as well as natural materials, including aqueous solutions and pastes to fabricate scaffolds for application in tissue engineering has been discussed. The rapidly growing field of bio-manufacturing faces significant challenges and opportunities. These problems, with the criteria to successfully design an implant are specified, along with future opportunities in the field.

Index Terms — Additive Manufacturing, Biomedical, Healthcare, Hydrogel, Rapid Prototyping technology, Tissue Engineering, 3D Dispensing Process.

I. INTRODUCTION

Complex diseases in medicine often demand time-consuming surgery. Surgical planning tries to minimize the duration of surgery to reduce the risk of complications. Normally, surgeons use imaging modalities like conventional Radiographs, Computerized Tomography and Magnetic Resonance Imaging. Such a visual representation of medical objects allows simulation of surgical procedures before surgery. The great advantage of Rapid Prototyping (RP) technologies is the precise reproduction of objects from a 3D medical image data set as a physical model, which can be looked at and touched by the surgeon. As it is well known, the term "rapid prototyping" refers to a number of different but related technologies that can be used for building very complex physical models and prototype parts directly from 3D CAD model.

Among these technologies are stereolithography (SLA), selective laser sintering (SLS), fused deposition modeling (FDM), laminated object manufacturing (LOM), inkjet based systems, and three dimensional printing (3DP). RP technologies can use wide range of biomaterials which gives possibility for their application in the medical field. The loss or failure of an organ or tissue is a frequent and costly problem in health care. The disparity between the need and availability of donor tissues has motivated the development of tissue engineering approaches aimed at creating cell-based substitutes of native tissues. Human tissue is well known to contain a considerable amount of water, the major emphasis of the state-of-the-art scaffold fabrication, supported by rapid prototyping (RP) technology, was placed upon melt and powder processing of biodegradable thermoplastics in the absence of water. In tissue engineering, the conversion from cell suspension into a three-dimensional (3D) organ structure is guided by 3D scaffolds because cells in cell 3D structure enables perfusion through interconnecting pores. The classification of bio-medical implants is as shown in Fig.1.

A key feature of RP technologies is the free-form fabrication (FFF) process: 3D computer models are cut into sequences of layers which are used to construct complex objects layer-by-layer. The layers are produced via solidification of melts, layer photo polymerization (stereolithography) or bonding of particles using either laser beam induced sintering (selective laser sintering) or special binders. Frequently among the different FFF technologies stereolithography (STL) is limited to the photo polymerization of toxic resins which limits the application in scaffold fabrication. In selective laser sintering (SLS) the materials are exposed to high temperatures which are prohibitive to many biodegradable and biofunctional materials. Biodegradable scaffolds prepared by FDM are currently being evaluated as carriers for osteoblasts. It appears likely that hard tissue replacement will be successful with FDM-based scaffolds. However, the processing window of FDM is rather narrow and restricts the application of...
bionanomaterials to the melt processing of PCL. Hydrogels were not applied in FDM processing. Also it should be noted that FDM and 3D printing of biodegradable polyesters is associated with the rapid formation of hydroxyacids during biodegradation, thus accounting for local cell toxicity. Recently, 3D dispensing in a liquid medium, also referred to as 3D plotting, was applied successfully to the RP of scaffolds and other complex 3D architectures using a remarkably large variety of synthetic and natural materials including melts, solutions, pastes, thermosets, filled polymers or reactive oligomers. One or two or more components can be applied in 3D plotting based on the dispensing technology, typical for one and two component adhesives and sealants. In addition to biocompatible and bioerodible polymers such as polyanhydrides, polyesters, polypeptides or polysaccharides, hydrogels find many applications in soft tissue cultures for tissue engineering applications. Hydrogels like gelatin, agar, fibrin or swollen collagen are used in simple scaffold structures as sheets, fibers, wovens or non-wovens. Hydrogels are advantageous because of their chemical similarity to the extracellular matrix, the flexibility, rapid diffusion of hydrophilic nutrients and metabolites, as well as their low content of dry mass, which causes reduced irritation and much lower level of degradation products. Typical hydrogels contain only around 0.5 up to 20 wt. % of dry polymer mass. The natural hydrogels are degraded readily because the entire polymer backbone is exposed to water-soluble enzymes. Chemical modifications such as increasing cross-link densities can be used to adjust biodegradation. The mechanical stability of hydrogels is often much lower than that of bulk materials and prohibits the use in stress-loaded implants. In view of the benefits of hydrogel scaffolds and their numerous applications in conventional scaffold fabrication, it is surprising that no successful attempts of RP technology combined with hydrogel chemistry are found in the literature. Here, we report an example of RP-based hydrogel scaffold fabrication using 3D plotting of thermoreversible gels in a liquid medium and combined with surface and pore modification.

![Classification of biomedical implants](image)

**Fig.1: Classification of biomedical implants.**

**II. MATERIALS**

Biomaterials are materials that interface with biological entities [1,2,3,4]. The National Institutes of Health Consensus Development Conference defined a biomaterial as “any substance (other than a drug) or combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body”[5]. A distinctive difference between a biomaterial over other materials is its benign coexistence with a biological system with which it interfaces [6]. The use of bio inert, bioactive materials (first generation of biomaterials) is an important response to the growing medical needs of a rapidly ageing population. Subsequently, the biomaterials field began to shift from bio inert materials to bioactive materials, which can elicit controlled actions and reactions within the body. Currently, four classes of biomaterials are used:

- Acellular tissue matrices(biological scaffolds)
- Metallic materials
- Ceramic material
- Polymers (naturally derived and synthetic polymers)

**2.1. Metals**

Metals and their alloys, due to their mechanical reliability, strength, stiffness, toughness and impact resistance, were used for load-bearing implants, such as hip and knee prostheses and fracture fixation wires, pins, screws, and plates. Metals have been used as parts of artificial heart valves, vascular stents, and pacemaker leads [7]. Important characteristics to be considered for medical applications are biocompatibility, appropriate mechanical properties, corrosion resistance and structural integrity [8]. Metallic biomaterials are classified as inert materials because they elicit minimal tissue response. In physiological environments, metals can suffer from corrosion, thus releasing ions, which may reduce biocompatibility and put at risk the use of implants. Major metals used in medical applications include commercially pure titanium and its alloys (a+b alloys, Ti–6Al–4V, Ti–Al– Nb and b-Ti alloys), cobalt-based alloys (Co–Cr–Mo, Co–Ni–Cr–Mo, Co–Cr–W–Ni), stainless steel (primarily type 316L), Ni–Ti alloys, Au-based materials, and Ag–Sn alloys [9,10,11].Titanium has one of the highest strength-to-weight ratios and corrosion resistance of metals. It has excellent biocompatibility due to its non-corrosive properties, low ion-formation tendency in aqueous environments and a dielectric constant comparable to that of water [12,13]. The material passivates itself in vivo by forming of an adhesive oxide layer.

**2.2. Ceramics**

Ceramics are inorganic materials with high compressive strength and biological inertness [14,15,16]. The most commonly used bioceramics are metallic oxides (e.g Al2O3, MgO), calcium phosphate (e.g., hydroxyapatite (HA), tricalcium phosphate (TCP), and octacalcium phosphate (OCP)), and glass ceramics (e.g. Bioglass, Cervital) [17,18]. Metallic oxides are considered to be nearly bio inert in biological environments, while calcium phosphate and glass ceramics can bond to bone when implanted. Bioceramics have been successfully used for hard tissue replacement due to their good biocompatibility and bioactivity. Their biocompatibility is a direct result of its chemical compositions, which contain ions commonly found in the physiological environment, such as Ca2+, K+, Mg2+, and Na+. Bone tissue becomes integrated into the bioactive ceramics through the biomineralization of a thin layer of calcium phosphate at the interface between the ceramics and the host bony tissue [19]. The interstitial body fluid is the very first medium of a bioactive ceramic interface after being hosted in a bony defect. The structure of the ceramic changes for the biomineralization of calcium phosphate by the interaction with the body fluid, which contains various proteins that must be significantly involved with
biomineralization [19,20]. This interfacial layer of calcium phosphate is almost independent of the ceramic type. Fig.2.

![Fig. 2: In vitro mechanism of formation of calcium phosphate on the surface of Na2O–CaO–SiO2 glass in SBF](image)

<table>
<thead>
<tr>
<th>Calcium phosphate compounds</th>
<th>Composition</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.05</td>
<td>Ostrich bone phosphate (OBP)</td>
<td>Ca10(PO4)6(OH)2</td>
</tr>
<tr>
<td>1.0</td>
<td>a-Tetracalcium phosphate (a-TCP)</td>
<td>Ca10(PO4)6(OH)2</td>
</tr>
<tr>
<td>1.0</td>
<td>c-Tetracalcium phosphate (c-TCP)</td>
<td>Ca10(PO4)6(OH)2</td>
</tr>
<tr>
<td>1.0</td>
<td>Hydroxyapatite (HA)</td>
<td>Ca10(PO4)6(OH)2</td>
</tr>
<tr>
<td>1.0</td>
<td>Tetralucite phosphate (TP)</td>
<td>Ca10(PO4)6(OH)2</td>
</tr>
</tbody>
</table>

Table.1

Illustrates the in vitro mechanism of the formation of calcium phosphate on the surface of a Na2O–CaO–SiO2 glass in SBF. The main calcium phosphate materials used for medical applications are indicated in Table 1. Synthethic hydroxyapatite (HA, Ca10(PO4)6(OH)2) is a bioactive material, with chemical characteristics similar to hard tissues such as bone and teeth, that promotes hard tissue ingrowth and Osseo integration when implanted into the human body [21,22]. The porous structure of this material can be tailored to suit the interfacial surfaces of the implant. As a bulk material, HA lacks sufficient tensile strength and is too brittle to be used in most load bearing applications [23]. In such cases, HA is coated onto a metal core or incorporated into polymers as composites [24]. HA is frequently used as a bioactive coating on hip prostheses. The ceramic coating on the titanium implants improves the surface bioactivity but often fails as a result of poor ceramic/metal interface bonding [25]. An alternative is the production of composite materials containing titanium and bioceramic as a reinforced phase. Due to the low bioreabsorbability of HA much attention has been paid to TCP ceramics [24,26]. Bioactive glasses, such as Bioglass, and A-W glass–ceramic have also been successfully used for tissue replacement [27,28]. Bioactive glasses stimulate the formation, precipitation and deposition of calcium phosphates from physiological solutions, enhancing the bone–matrix interface strength. Bioglass is bioactive with low fracture toughness [28,29], while the bioactive A-W glass–ceramic has excellent mechanical properties and high bioactivity (higher than HA), being clinically used for iliac and vertebral prostheses and intervertebral spacers [30]. It was observed that ionic dissolution products from Bioglass and other silicate-based glasses stimulate gene expression of osteoblasts.

### 2.3. Polymers

Polymers for medical applications can be naturally derived or synthetic, the latter of which can be biodegradable or bio inert. Bioinert synthetic materials include polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP), polymethylmethacrylate (PMMA), polystyrene (PS), polytetrafluoroethylene (PTFE), polyesters, polyamides (PA-nylon), polyurethanes (PUR), and polysiloxanes (silicone) [31,32]. Biodegradable synthetic polymers include poly (glycolic acid), poly (lactic acid), their copolymers, and poly (p-dioxanone). Natural polymers include albumin, collagen, cellulose, hyaluronic acid, starch, chitosan, dextran, silk, heparin, and DNA [31,32]. Biodegradable synthetic polymers have been used in a number of clinical applications, such as resorbable sutures, drug delivery systems, and orthopedic fixation devices such as pins, rods and screws, and scaffolds for tissue engineering.

### 2.4. Composites

A wide range of polymer-based composite materials were developed and investigated for biomedical applications. These materials can be classified as shown in Fig.3 [33]. A composite material made of an a vital (non-living) matrix and reinforcement phases is called an “avital/avital” composite. Alternatively, a composite material comprising a vital (living) and avital (non-living) material is called a “vital/avital” composite [33].

![Fig. 3: Classification of polymer-based composite biomaterials](image)

### 2.5. Hydrogels

Hydrogels are cross-linked hydrophilic polymers that exhibit excellent biocompatibility, causing minimal inflammatory responses, thrombosis, and tissue damage. Hydrogels can also swell large quantities of water without the dissolution of polymer due to their hydrophilic and cross-linked structure which gives them characteristics similar to soft tissues. They also present high permeability for oxygen, nutrients and high water-soluble metabolites, generally used above their glass transition temperature. Hydrogels can be chemically tailored to respond to certain environmental stimuli, as so-called temperature-responsive, potential-specific and pH-sensitive gels (Fig. 4). They are extensively used in medicine for applications such as contact lenses, biosensors, linings for artificial implants, wound healing, and drug delivery devices. In wound healing applications, hydrogels protect the wound from desiccating, thus providing control of the wound surface hydration, sometimes absorbing excess exudate and often providing moisture. Hydrogel formation can be achieved by chemical reaction during feeding coreactive components in two-component dispensers or by plotting one component in a liquid medium containing a coreactive component.
Alternatively, single-component thermo reversible hydrogels can be plotted at elevated temperatures into a liquid medium with temperature below the gelation temperature. The gelation of thermo reversible hydrogels is not an exothermic process like crystallization. This fact is favorable because of the rapid gel formation without delay and local overheating. The gelation depends only on the temperature of the material.

### III. MANUFACTURING PROCESSES

Additive electro-chemical and physical processes, through which physical objects are created from computer-generated models, emerged in the 1980s. The basic concept of additive fabrication is layer laminate manufacturing, in which 3D structures are formed by laminating thin layers according to 2D slice data obtained from a 3D model. The main advantages of additive electro-chemical and physical techniques are the capacity to rapidly produce very complex 3D models and the ability to use various raw materials. When combined with clinical imaging data, these fabrication techniques can be used to produce constructs customized to the shape of the defect or injury. Some processes operate at room temperature, thus allowing cell encapsulation and biomolecule incorporation without significantly affecting viability. This section describes the most relevant additive electro-chemical and physical processes either commercially available or under development.

#### 3.1. Electrospinning

Electrospinning is the most relevant electro-chemical process to produce nano-scale meshes for tissue engineering [34,35,36,37]. It is a simple and versatile process by which nanofibers with diameters ranging from a few nanometers to several micrometers can be produced using an electrostatically driven jet of polymer solution (solution electrospinning) or polymer melt (melt electrospinning). The basic requirements of an electrospinning apparatus are shown in Fig. 5 including: (1) a capillary tube with a needle or pipette, (2) a high-power voltage supply, and (3) a collector or target. This collector can move in the vertical direction, enabling electrospinning as an additive technology. Electrical wires connect the high-power supply to both the capillary tube, which contains a polymeric solution, and the target. Melt electrospinning requires, the polymeric jet to be cooled, while solution electrospinning relies on the evaporation of the solvent to produce fibres.

Initially, as a result of surface tension, pendant droplets of the solution are held in place. A conical protrusion, known as a Taylor cone, is formed when a critical voltage is applied to the system. An approximately straight jet emerges from the cone but it cannot stand for long. The jet then emerges into a diaphanous and conical shape. The conically moving jet experiences bending instabilities and is directed towards the collector, which has the opposite electrical charge. The solvent evaporates, and dry polymer fibres are deposited until the jet reaches the collector.
Materials commonly used in electrospinning include:

Solution electrospinning: Polyethylene-co-vinyl acetate (PEVA/PLA), Polylactic acid (PLA), Polyvinyl alcohol (PVA), Polyacrylonitrile (PAN), Polycarbonate (PC), Polybenzimidazole (PBI), Polyurethanes (PU), Nylon6,6 (PA-6,6), Polyethylene oxide (PEO), Collagen/PEO, Polyethylene terephthalate (PET), and Polyethylene naphthalate (PEN), PET/PEN.

3.2. 3D printing

3DP technology creates 3D physical prototypes by solidifying layers of deposited powder using a liquid binder. The machine spreads a layer of powder from the feed box to cover the surface of the build piston. The printer then prints binder solution onto the loose powder, forming the first cross section. For multi-colored parts, each of the four print heads deposits a different color binder, mixing the four colour binders to produce a spectrum of colours. The powder is glued together by the binder at where it is printed. The remaining powder remains loose and supports the following layers that are spread and printed above it. When the cross section is complete, the build piston is lowered and a new layer of powder is spread over its surface and the process is repeated. The part grows layer by layer in the build piston until the part is complete. The part is surrounded and covered by loose powder. The build piston is raised and the loose powder is vacuumed away revealing the complete part.

3DP [38] was employed with particulate leaching to create porous scaffolds, using polylactic-co-glycolide (PLGA) powder mixed with salt particles and a suitable organic solvent. The salt particles were leached using distilled water. Cylindrical scaffolds measuring 8 mm (diameter) by 7 mm (height) with pore sizes of 45–150 mm and 60% porosity were fabricated. Hepatocytes were successfully attached to the scaffolds. The influence of pore size and porosity on cell adhesion and proliferation were investigated. Disc shaped poly (L-lactic acid) (L-PLA) scaffolds measuring 10 mm (diameter) by 2 mm (height) were produced through both 3DP and salt and leaching methods. The scaffolds were produced with two different porosities (75% and 90%) and four different pore size Distributions (<38, 38–63, 63–106 and 106–150 mm), and tested with cell cultures using canine dermal fibroblasts, vascular smooth muscle cells and microvascular epithelial cells.

A modified thermal inkjet printer and demonstrated the feasibility of printing microvasculature with human microvascular endothelial cell suspension in thrombin solutions onto fibrinogen solutions, which served as the substrate. The printed cells achieved the capacity to interact and proliferate within fibrin channels forming a tubular lining. An alternative process which was developed is the concept of cell printing. This process prints gels, single cells and cell aggregates offering a possible solution for organ printing. To be used for cell printing, the thermal or piezotip printheads and ink cartridges are modified to allow bioinks to be printed. These bioinks usually consist of aqueous media, thermoreversible polymers, or polymer/hydrogel precursors combined with living cells. Laser-assisted cell-printing techniques have also been developed. These techniques comprise the so-called laser guidance direct write (LG DW), laser-induced forward transfer and modified laser-induced forward transfer processes (Fig.8). The LG DW process was the first reported technique to print viable cells by forming patterns of embryonic-chick spinal-cord cells on a glass slide. Shortly after this, modified laser-induced forward transfer techniques (LIFT) and modified inkjet printers were also used to print viable cells and proteins, followed by the recently introduced electrohydrodynamic jetting (EHDJ) method.

3.3 Stereo lithography

The technique builds three-dimensional models from liquid photosensitive polymers that solidify when exposed to ultraviolet light. The model is built upon a platform situated just below the surface in a vat of liquid epoxy or acrylate resin. A low-power highly focused UV laser traces out the first layer, solidifying the model’s cross section while leaving excess areas liquid. Next, an elevator incrementally lowers the platform into the liquid polymer. A sweeper re-coats the solidified layer with liquid, and the laser
Electron Beam Melting) to form cylinders. In SLS, a laser beam is traced over the surface of a tightly compacted powder made of thermoplastic material. The laser melts the powder where it strikes, and SLS is also used to produce surgical guides for tissue engineering applications. Stereolithographic processes have been used to produce hearing aids, micro needles for transdermal drug delivery and scaffolds for tissue engineering with or without encapsulated cells (Fig. 10). Stereolithography is also used to produce surgical guides for the placement of dental implants, temporary crowns and bridges and resin models for lost wax casting.

Fig. 10: Mushroom-shaped cap of a hearing aid

3.4 Selective Laser Sintering and Melting process

Selective laser sintering (SLS) and selective laser melting (SLM) are additive manufacturing processes that use high-energy light sources to consolidate powder material. In SLS, a laser beam is traced over the surface of a tightly compacted powder made of thermoplastic material. The powder is spread by a roller over the surface of a build cylinder. A piston moves down one object layer thickness to accommodate the layer of powder. The powder supply system is similar in function to the build cylinder. It also comprises a cylinder and piston. In this case the piston moves upward incrementally to supply powder for the process. Heat from the laser melts the powder where it strikes under guidance of the scanner system. The CO2 laser used provides a concentrated infrared heating beam. The entire fabrication chamber is sealed and maintained at a temperature just below the melting point of the plastic powder. Thus, heat from the laser needs to only elevate the temperature slightly to cause sintering, greatly speeding up the process. A nitrogen atmosphere is also maintained in the fabrication chamber which prevents the possibility of explosion in the handling of large quantities of powder.

Contrary to SLS, SLM uses high-powered laser beams to directly create 3D metal parts by fusing very fine metallic powders. Table 3 summarises the major advantages and disadvantages of SLM to process metal powders. SLS and SLM have been used to produce both permanent and temporary implants. The use of SLM to produce porous titanium constructs with complicated internal structures for bone ingrowth applications were explored. The constructs were produced using Ti powder of less than 45 mm particle size. The compressive strength was in the range of 35–120 MPa when the porosity was in the range of 75–55%. Porous Ti constructs were subjected to NaOH, HCl, and heat treatment to provide bioactivity. Treated constructs formed bone-like apatite on their surfaces in a stimulated body fluid within 3 days. In vivo research also showed that new bone penetrated into the pores.

![Table 3: Main advantages and disadvantages of SLM](image)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>No distinct binder and melt phases</td>
<td>Not suitable for well-controlled composite materials (e.g. WC-Co)</td>
</tr>
<tr>
<td>Elimination of time-consuming and costly furnace post-treatments for debinding, infiltration or post-sintering</td>
<td>High laser power and good beam quality (expensive lasers); smaller scanning velocities (longer build times)</td>
</tr>
<tr>
<td>Suitable for producing fully dense parts in a direct way</td>
<td>Melt pool instabilities and higher residual stresses</td>
</tr>
</tbody>
</table>

SLM is also used to produce a wide range of Ti–6Al–4V medical implants, ranging from cylinders with regular porosity to a human vertebra model (Fig. 12). In vitro studies were performed with porous structures using human osteoblasts. Cell spreading and proliferation was observed. Similar studies were performed at the University of Leuven: some ten different scaffold geometries were produced and seeded with human periosteum-derived cells. After 14 days of culture in a growth medium (GM based on DMEM and bovine serum) and osteogenic medium (OM = GM + dexamethasone + ascorbic acid), the cells were found to be viable and proliferating in all scaffolds. GM culture resulted in more cells and a greater extend of pore occlusion.

![Fig. 12: Examples of Ti–6Al–4V parts.](image)

3.5 Electron beam melting process

EBM (Electron Beam Melting) is an additive manufacturing process that uses an electron beam to scan a layer of metal powder
on a substrate, forming a melt pool. The system consists of the electron beam gun compartment and the specimen-fabrication compartment both kept in a high vacuum. Advantages of using EBM over SLM include very small spot sizes, very high beam-material coupling efficiency, high scanning speed and beam deflection without the use of moving mirrors. The accuracy of EBM is in the range of 0.3–0.4 mm, and the surface finish tends to be rough, with a Ra value in the range of 25 mm. Table 6 compares EBM and SLM.

<table>
<thead>
<tr>
<th>Comparison between EBM and SLM</th>
<th>EBM</th>
<th>SLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal source</td>
<td>Electron beam</td>
<td>Laser</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>Vacuum</td>
<td>Gas</td>
</tr>
<tr>
<td>Scanning</td>
<td>Deflection coils</td>
<td>Galvanometers</td>
</tr>
<tr>
<td>Energy absorption</td>
<td>Conductivity-limited</td>
<td>Absorptivity-limited</td>
</tr>
<tr>
<td>Powder pre-heating</td>
<td>Use electron beam</td>
<td>Use infrared heaters</td>
</tr>
<tr>
<td>Scan speed</td>
<td>Very fast, magnetically driven</td>
<td>Limited by galvanometers</td>
</tr>
<tr>
<td>Energy costs</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Surface finish</td>
<td>Moderate to poor</td>
<td>Excellent to moderate</td>
</tr>
<tr>
<td>Feature resolution</td>
<td>Moderate</td>
<td>Excellent</td>
</tr>
<tr>
<td>Materials</td>
<td>Metals (conductors)</td>
<td>Metals, ceramics and polymers</td>
</tr>
</tbody>
</table>

Table 4

EBM has been used to produce titanium root-form implants (Ti–6Al–4V ELI), femur hip implants, dental implants and knee replacement implants (Figs. 13 and 14). Evaluations of the in vivo performance of Ti–6Al–4V ELI dental implants fabricated via EBM and its comparison to a commercially available porous-coated press-fit dental implant (Endopore, Innova Corp, Toronto, Canada) were carried out. Cylindrical shaped implants 3 mm 5 mm long were implanted in a rabbit tibia and retrieved after 6 weeks postoperatively. Histology results revealed osteo-integration of surrounding bone with both implant types, suggesting that the implants produced by EBM perform equally well as commercial implants.

![Fig.13](image1.png)

Fig.13: (A) Co–29Cr–6Mo femoral prototype with mesh structure produced by EBM, HIP-annealed using ASTM-F75 standard, machine finished and partially polished, (B) magnified view of the mesh structure section .

3.6 3D Dispensing Process (3D Plotting)

This process is used widely in the manufacture of hydrogels. Hydrogel formation can be achieved by chemical reaction during feeding coreactive components in two-component dispensers or by plotting one component in a liquid medium containing a coreactive component. Alternatively, single-component thermo reversible hydrogels can be plotted at elevated temperatures into a liquid medium with temperatures below the gelation temperature. The gelation of thermo reversible hydrogels is not an exothermic process like crystallization. This fact is favorable because of the rapid gel formation without delay and local overheating. The gelation depends only on the temperature of the material.

Among many thermoreversible hydrogels agar and gelatin are used due to their well-known biocompatibility. The standard 3D dispensing machine was modified to the demands of temperature dependent gelation. A metal double jacket cartridge was combined with a thermostat to achieve dispenser temperatures up to 100°C. The main problem of the plotting of thermoreversible hydrogels is associated with the low viscosity of the aqueous polymer solution before the gelation. This can cause collapse of 3D architectures due to gravity force induced flow subsequent to plotting. In order to circumvent this problem, the plotting was performed in a liquid medium. This medium has approximately the same density as the plotting material. The result is a compensation of the gravity forces by means of buoyancy forces. In the case of thermoreversible hydrogels the temperature of the medium is below the gelation temperature of the hydrogel. Fig. 3 displays the 3D plotter used to position micro strands in an aqueous plotting medium.

![Fig.14](image2.png)

Fig.14: Ti–6Al–4V ELI acetabular cups.

![Fig.15](image3.png)

Fig.15 Calculated structures of a scaffold produced by 3D plotting: (a) one layer, (b) two layers. The repeat unit in the layer (d2) and between the layers (d3) is indicated.

In this process, the polymer solution is forced through the cartridge nozzle by applying air pressure. The moving of the dispensing unit as well as the air-pressure feed valve is computer controlled by a modified CNC-software. The plotting material leaves the nozzle
and solidifies in the cold plotting medium immediately after bonding together with the previously plotted strand. Bonding by interdiffusion represents one of the prime requirements and early gelation prior to contact must be prevented. Therefore, the temperature of the plotting material in the double jacket cartridge is set significantly higher with respect to the gelation temperature, in order to delay the gelation for a few seconds. In contrast, the temperature of the medium is set to be only a few degrees below the gelation temperature. If the delay is too long, the plotting material, which exhibits low viscosity, will continue to flow and to deform the plotted structure. This sensitive balance depends on the amount of material leaving the nozzle, on the moving speed of the plotting head, and on the thickness of each layer. Choosing the right temperature conditions also prevents the nozzle from blocking. High viscosity of the plotting medium is preferred for the stability of the process. The diffusion of the polymer chains of the plotting material into the plotting medium has to be prevented. This can be achieved by means of fast gelation or by using a plotting medium which does not dissolve the polymer. Especially a medium with a complete different polarity has sometimes advantages because it improves the adhesion between the hydrogel layers. High differences in polarity enlarge the surface tension sometimes too much, thus affording droplet formation instead of continuous dispensing. Nevertheless it was found that, aqueous gelatin solution is a good medium for the plotting of agar scaffolds due to its high viscosity, its density and its excellent biocompatibility.

Agar gel is an ideal material for 3D plotting of scaffolds because the gel is stable at room temperature and 37°C is not cell-toxic and exhibits adequate mechanical stability. The gelation process is fast and reliable and therefore easy to control. The gelation temperature is in the range of the temperature of an incubator which possibly allows incorporation of human cells into the 3D plotting process in future. Purified types of agars are used as matrix for the cloning of cells. But the lower gel point is accompanied with lower gel strength. Gelatin has a gelation temperature of 20°C and forms a weak gel. The gel is not stable at 37°C. Therefore, it cannot be used as a scaffold and would require further chemical modification.

Fig. 17: Swelling of different concentrated cylindrical agar samples after 1 week: (a) in demineralized water, (b) in 0.9% aqueous NaCl.

Formation of a thermoreversible hydrogel does not affect the density. Therefore, it is sufficient to measure the density and swelling of the hydrogel. The swelling of agar hydrogels was determined in demineralized water and isotonic NaCl-solution. Fig. 17 a–b displays the results. The swelling ratio is and of the samples in demineralized water is between 1.02 and 1.03. This might be a problem for the plotting of layers with a constant thickness. The situation in isotonic NaCl-solution is different. The gel shrinks at low agar concentrations and remains nearly unchanged at concentrations of 3–5% agar.

Another important aspect is related to the swelling of the plotting material during plotting and during cell culture. The swelling during processing must be prevented, because it is very important for the layered manufacturing process that each layer remains exactly at the same location until the whole object is finished. Swelling during the usage of the scaffolds is not desirable because it changes the defined structure, but does not cause destruction of the scaffold. Furthermore, the bottom of the plotting medium vessel should be rough and sticky enough to maintain a good adhesion of the first hydrogel layer over the entire plotting process. The polarity of the bottom has to be similar to that of the plotting material. Sandblasted metal plates proved to be ideal for this purpose. It is obvious that many parameters influence this plotting technique. The most important ones are the thermal behavior of the plotting material, its viscosity; its swelling in the plotting medium and its density.

Another important parameter is the density of the hydrogel material (Fig. 18), which determines the density of the plotting medium. The density of agar solutions rises from 0.998 (distilled water) up to 1.025 g/cm3 (6% solution). The ideal plotting
medium has nearly the same density as the plotting material. An aqueous solution of gelatin (4%) in isotonic water suits the demands for the plotting medium in the best way. Plotting material was a buffered isotonic 4–5% agar solution. The temperature of the plotting material should be 50–70°C (lower temperatures for agarose) and the temperature of the plotting medium 20°C. Gelatin solution requires a cartridge temperature of 40°C and a medium temperature of 51°C. The desired hydrogel scaffolds were produced by using this knowledge about the plotting process and the properties of the materials. The structure in Fig. 19 was produced by 3D plotting of a agar and exhibits the desired 3D pores.

3.7 Summary

Table 5 summarises the actual state of the art of biomaterial processing with appropriate electro-chemical and physical additive manufacturing for the fabrication of both temporary and permanent implants. However, this is an extensively researched area, and new options both in terms of materials and processes are continuously emerging.

IV. Conclusions and future challenges

Recent investigations with additive electro-chemical and physical processes demonstrated the potential to fabricate customised permanent and temporary implants. A wide range of biocompatible materials is available, ranging from metals and metallic alloys to ceramics and polymers, including hydrogels and SMPs. Several techniques also show potential for processing composite materials that combine synthetic materials and biological ones, such as cells, proteins and growth factors. However, the use of additive electro-chemical and physical processes in the medical field are still in its early life. Also, the very versatile 3D plotting process, involving 3D dispensing in a liquid medium with matched density and polarity, offers attractive opportunities for manufacturing hydrogel scaffolds. The 3D plotting process depends on various parameters such as the ratio of gelation temperature and plotting temperature, the nozzle type, the densities of the plotting material and the plotting medium as well as on their rheological behavior. Although significant progress was made in hydrogel processing by means of RP technology, more basic research is required aiming at improving the performance of hydrogel scaffolds in tissue engineering. Further-more, the 3D plotting technology also offers the possibility of hydrogel formation via reactions occurring during mixing of individual components or via reactions occurring during plotting in the plotting medium. Especially the 3D plotting of fibrin, collagen or polyelectrolytes is of interest for scaffold fabrication and tissue engineering. Longer growth time of cells on the hydrogel scaffold would answer the question whether the grown cells

![Fig.19: Images of an agar scaffold](image-url)
form a new tissue or not and how the structure of the new tissue would be.

The rapidly growing field of biomaterials faces significant challenges and opportunities. Relevant challenges to be addressed in the future include:

- Establishing a directory materials and related processes and assembly techniques.
- Applying both nano and micro technologies for enhancing efficacy and precision.
- Standardising processes, design and metrology tools.
- Achieving a fundamental understanding of manufacturing processes and convergence of techniques for best and affordable health care.
- Development of in situ manufacturing strategies, such as in situ tissue engineering.
- Enhancing multidisciplinarity, linking clinicians and engineers to facilitate further developments and the clinical translation of the products/systems being investigated.
- Packaging, handling, transportation and accurate tracking, and deploying of biomaterialized parts and their building blocks.
- Scaling up additive electro-chemical and physical processes towards clinical application.
- Other thermoreversible hydrogels, e.g., protein-based systems, should be investigated.
- Research aiming at implementing living cells into the 3D plotting process and the use of different cartridges, which can be changed during 3D plotting and containing different cell types must be carried out.
- Mechanical strength and the microporosity of the scaffolds have to be optimized in future.
- The software compatibility to the CAD-software and the import of data from computer tomography are essential for a later clinical use and has to be refined to improve CAD capabilities.

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VI. References


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