The Pharma Innovation Journal 2021; SP-10(12): 01-05

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 01-05

www.thepharmajournal.com
Received: 01-10-2021
Accepted: 03-11-2021

TV Meenambigai
Vaccine Research Centre Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

C Sworna Kumari
Vaccine Research Centre Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Corresponding Author
TV Meenambigai
Vaccine Research Centre Viral Vaccines, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

An outlook into the novelties of bioprinting technology

TV Meenambigai and C Sworna Kumari

Abstract
Bioprinting involves computational modeling, utilizes biomaterials, cells, and bioactive components as a "bioink" to fabricate prospective tissue structures in three-Dimensional (3D) printing. The printed product is heavily influenced by biomaterial parameters such as biocompatibility, cell viability, and the cellular microenvironment. Bioprinting provides control over cell placement and therefore creates a homogenous distribution of cells correlating to a uniform tissue in growth. This technology has already found success in human studies and developing in veterinary research, where a variety of functional tissues have been generated for both *in vitro* and *in vivo* applications. Various bioprinting technologies using Computer-Aided Design has been progressed in printing different types of tissue, including vasculature, heart, bone, cartilage, skin and liver. The development of such 3D *in vitro* systems has attracted increasing attention in human and veterinary healthcare predominantly driven to rectify the demand of a limited supply of organs and a demand for less expensive drug testing models.

Keywords: bioprinting, bio-ink, drug testing, applications

Introduction
Bioprinting is defined as the printing of structures in 3-dimensional outlook using living cells, active biomolecules and biomaterials generally named as bioinks. Developmental biology, stem cell science, chemistry, computer science, and materials science are all needed in bioprinting and hence is an interdisciplinary area. Bioprinting has made huge strides towards the printing of organs using Computer-Aided Design/Computer-Aided Manufacturing (CAD/CAM) technology leading to an enhanced fabrication process for Additive Manufacturing (AM), known as Three-Dimensional (3D) printing. Additive manufacturing (AM), fabricates by depositing material layer-by-layer according to a digital model. Bioprinting utilizes biomaterials, cells, biomaterial and bioactive components as a "bioink" to fabricate prospective tissue structures in three-Dimensional (3D) printing. The printed product is heavily influenced by biomaterial parameters such as biocompatibility, cell viability, and the cellular microenvironment. Bioprinting provides control over cell placement and therefore creates a homogenous distribution of cells correlating to a uniform tissue in growth. Various bioprinting technologies using Computer-Aided Design (CAD) has been progressed in printing different types of tissue, including vasculature, heart, bone, cartilage, skin and liver. The development of such 3D *in vitro* systems has attracted increasing attention in human and veterinary healthcare predominantly driven to rectify the demand of a limited supply of organs, demand for less expensive drug testing models and cancer studies. The ultimate goal is to develop implantable organs and tissues to replace autografts, which are associated with donor site morbidity and necessitate too invasive surgeries. A Bioprinted tissue recreates the complexity and heterocellularity of native tissues. The primary and most common bioprinting techniques, fundamentals, formulations and properties of the bioinks and cell sources are explained below with current commentary on the applications and limitations of bioprinting technologies used for tissue engineering applications, human medicine and veterinary research.

Bioprinting process
In general, the process of bioprinting 3D tissues is divided into three major steps:
1. Pre-bioprinting, 2. Bioprinting 3. Post bioprinting.

Pre-bioprinting
The goal of this step is to generate a 3D tissue or organ model that can be created using medical imaging technology or Computer-Aided Design (CAD). The most common imaging techniques are X-ray, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI)

which are utilized to provide information of the tissue or organ on the anatomical structure. Some engineering software designed as 3D model into horizontal cross-sectional layers creating stereolithography data, that is then utilized in 3D-bioprinting for layer-by-layer stereolithographic accumulation to fabricate a 3D physical mode (Colin *et al.*, 2021) [3].

Bioprinting

The development of bioink for bioprinting of the tissue construct is the next step. Bioink is a cell-laden fluid material that may contain biomaterials, cells, growth factors, microcarriers, etc. The development of appropriate bioink is a critical step in the successful application of bioprinting. Printability, biocompatibility, cell viability, and mechanical properties of the bioink are the critical step on the printed tissue construct.

Post-bioprinting

The post-bioprinting maturation process, which usually takes place in bioreactors, is a critical step for developing functional bioprinted constructs via both physical and chemical stimulation. (Jipeng *et al.*, 2016) [9].

Bio-inks

Bio-inks are biomaterial solutions containing the living cells and are so important to protect the cells against stressors during the printing process.

Parameters of biomaterials

The physical characteristics of biomaterials determine the optimal printing type. Low- viscosity materials are the best example for bioprinting because cells can grow well in the low-pressure environment. (Khaitwala *et al.*, 2012). Other material properties, such as pore size and interconnectivity, also influence the encapsulated cells.

Biocompatibility

Biocompatibility is the first parameter to be considered when fabricating scaffolds. Scaffold materials must accommodate the encapsulated cells and the recipient's body, only then the implant will be cytocompatible and support cell growth, attachment, proliferation and migration preventing severe inflammation or immunologic rejection. (Nakamura *et al.*, 2005) [17].

Porosity and interconnectivity

The behavior of cells after adhesion to the scaffold is mainly affected by the Pore shape, volume, size and geometry. Different pore sizes in matrices can influence extracellular matrix development as they are strongly linked to cellular organisation, collagen I assembly, and mineralization. Porosity and interconnectivity play important roles in the growth of surrounding tissues. Open and interconnected pores can allow oxygen and nutrients to be transported into the interior and eliminate the waste generated by cellular metabolism (Matsiko *et al.*, 2015) [13].

Mechanical properties

Physical parameters are an essential component of tissue engineering scaffolds, especially for the regeneration of hard tissues like bones and cartilages with appropriate mechanical strength as that of natural bones. When artificial bones with high elasticity are implanted in situ, they may cause stress shielding and obstruct new bone formation. (Lou *et al.*, 2015) [12] Most material components of bioink are used in tissue engineering and limit the application of printed scaffolds. They must have appropriate hydrophilicity, pH neutrality, and degradability without the formation of toxic macromolecules, in addition to good biocompatibility, high porosity, and matching mechanical properties. (Nadeem *et al.*, 2015) [16]. The four common bio- ink materials are hydrogels, microcarriers, cell aggregates, and decellularized matrix components.

Hydrogels

Hydrogels are the best attractive materials used in bioprinting because they have three- dimensional network of polymer chains holding a mass of water. For the processing of bioprinting, the hydrogel should form a polymer network in the physical junctions between hydrogel macromolecules. Some photo-initiators and monomers during hydrogel crosslinking affects cell viability depending on radical concentration and the length of exposure. So the biocompatible hydrogels are used for fabrication in bioprinting technology. (Nicodemus and Bryant 2008, Mohsen *et al.*, 2021) [18, 14].

Cell aggregates

Cell aggregate configurations provide a scaffold-free bio-ink alternative. Cell–cell interactions are of crucial importance in tissue formation. The type-I transmembrane protein cadherin causes multicellular aggregates for tissue morphogenesis. Cadherin allows intercellular adhesion which is important for cell–cell communication. Tissue spheroids are spherical cell aggregates that range in size from 200 to 400 µm. They can be used as building blocks for tissue engineering or as tissue models for pharmaceutics. The self-assembling cellular spheroids mimic developing tissue through fusion and reorganization. Most cells donot spontaneously aggregate in culture, rather they have to be induced to aggregate by some means. Different techniques exist for the generation of tissue spheroids, the most common of which uses cell-adhesion inert hydrogel moulds in which thousands to millions of cells are cultured in micro-wells for 24–28 hours. At the bottom of the well, cells adhere to each other and form spheroids due to radial contraction and cadherin-mediated cytoskeletal reorganization.

The hanging drop method uses gravity to concentrate the cells in one spot for cell aggregation. It is a simple technique that only requires a tissue culture plate and a cell suspension. A small drop of the cell suspension is pipetted on the plate, which is then inverted to make the droplet hang.(Junjie *et al.*, 2020) [10] Tissue strands are cylindrical mini-tissues that can be used to produce tissues through bioprinting approaches. Semi-permeable tubular alginate capsules were used to allow exchange between cells and the medium for nutrients and oxygen supply. Alginate capsules were extrusion-printed through coaxial printing of sodium alginate and a crosslinker solution. In alginate capsule Cell pellets were injected until the capsule was completely filled. The ends of the capsule were blocked during 5–7 days of culture for the aggregation of cells. After that, the alginate capsule was decrosslinked, leaving the tissue strand. A heterocellular tissue strand could be fabricated through an overnight co-culture with a secondary cell type and fibronectin. (Ilze *et al*. 2017) [18].

Decellularization

Decellularization is a novel technique used in tissue engineering to create scaffolds made of biologically relevant ECM components. Decellularization is the process of removing the cellular components of a donated organ while keeping the ECM components. The dECM provides site-specific mechanical and biochemical interactions that guide cell adhesion, proliferation, and differentiation. (Park *et al.*, 2018) [20].

Decellularization can be accomplished through chemical, physical, or enzymatic means. It was previously thought to be an alternative to 3D bioprinting; however, recent efforts have converged these two technologies by using dECM as a bioink. dECM can be altered to form a soft, gel-like material that can be loaded into a 3D bioprinter. Because dECM bioinks retain native ECM components, they aid in tissue regeneration and cell stabilisation, as well as facilitating favourable tissue organisation and remodelling. Although dECM bioinks can mimic native tissue environments and provide construct stability, dECM bioink alone lacks the mechanical strength required to develop load-bearing tissues, necessitating the use of other materials and require scaffolds for additional support. (Garreta *et al.*, 2017) [7].

Microcarriers

Microcarriers, which are supportive matrices, can be added to bioink formulations to increase cell density and provide structural support. Microcarriers can be made of either synthetic or natural materials, such as plastic and glass, or cellulose and gelatin.

These spherical shape structures possess interconnected pores ranging from 60 to 400µm in size. By modulating cell shape and organisation, these characteristics promote efficient cell adhesion, robust cell proliferation, and differentiation. Derby (2012) [6] defines formalised microcarriers as substrates for anchorage-dependent cellular adhesion and help printed cells maintain their phenotypic stability. Their spherical structure improve the gas and nutrient transfer and results in a large surface area for viable cell attachment. However, microcarriers have limitations such as limited scalability and formation of toxic products. Hence there is a need for an effective detachment system. (Jipeng *et al.*, 2016) [9].

Bioprinting Technology Droplet-based bioprinting

Droplet-based bioprinting (DBB) is a simple and agile technique with biologics which can be deposited in a precise and controlled way. Initially droplets (Picolitre) are layered on top of a substrate without contact between the nozzle and the substrate. DBB are highly versatile, compatible with many biological materials, which prints with low viscosities (3.5–12 mPa s−1), and enables high speed and high resolution. However, it faces some challenges like non uniform droplets and inconsistent encapsulation of cells. DBB can be subdivided into three categories; inkjet, acoustic, and micro-valve bioprinting. (Ilze *et al.*, 2017) [8].

Inkjet bioprinting

Researchers began 3D bioprinting by modifying standard 2D inkjet printers to print bioink in successive layers. Inkjet printers work by depositing droplets of ink at precise points on a substrate. Thermal, piezoelectric, or electromagnetic forces can be used to expel droplets from the reservoir nozzle. Despite the fact that these forces produce local extreme conditions, the transient nature of the pressure allows the cells to maintain viability with minimal stress. Bioprinting with inkjet printers can be advantageous due to high-speed, availablility and relatively low-cost technology (Boland *et al.*, 2006) [1].

The extracellular microenvironment which contains a variety of stimuli, including physical, chemical, and biological factors, that direct cell adhesion, proliferation. Bioprinting technologies such as inkjet printing have successfully deposited biological molecules such as proteins and nucleic acids. An advantage of inkjet printing is the ability to control the concentration gradient, measuring the molecular patterning of growth factors such as BMP2, epidermal growth factor (EGF) and fibroblast growth factor 2. To print 3D artificial tissues, studies of 2D molecular arrays may provide clues about the function of growth factors in their niche. (Murphy and Atala 2014) [15]. Disadvantage of using inkjet printers is that special considerations must be made in bioink selection. Because the ink must be emitted at a high rate from a small diameter nozzle, low viscosity is critical. Even when using the best bioink for an inkjet printer, clogging can occur. Furthermore, when using stem cells, passing cells through a high-pressure bottle neck may have an effect on cellular function, including possible pressure to differentiate into a specific lineage. (Cui *et al.*, 2010, Cui *et al.*, 2012) [5, 4].

Microextrusion bioprinting

Microextrusion based bioprinting, also called as Fused Deposition Modelling (FDM), is an additive manufacturing process that involves around the deposition of a single near-continuous stream of material in layers to form the desired three-dimensional structure. The reservoir's pressure can be supplied by a variety of mechanical devices, the most common of which are pneumatic / mechanical pistons and screw drive mechanisms. The bioink that must be used is the most significant distinction between microextrusion and inkjet deposition. (Patrick rider *et al.*, 2018) [22].

In comparison to inkjet and laser-assisted bioprinters, microextrusion bioprinters have the advantage of being able to work with a wider range of viscous bioinks. Although viscous inks may be used, the high pressures generated when printing these inks can have an effect on the cells. Even when cells are able to withstand the high shear forces of extrusion-based printing, they may lose viability or be mechanically stimulated to differentiate abnormally. Because both of these technologies use small diameter nozzles, nozzle clogging can be an issue, as it is with inkjet bioprinting. Certain techniques, such as frequent cleaning and capping of the nozzle when not in use, can help prevent this issue. (Pati *et al.*, 2015; Ozbolat and Hospodiuk 2016) [21, 19].

Laser assisted bioprinter

Laser-assisted bioprinting (LAB) deposits biomaterials onto a substrate using a laser as the energy source. A pulsed laser source, a ribbon coated with liquid biological materials deposited on a metal film, and a receiving substrate are typically used in this technique. The lasers irradiate the ribbon, causing the liquid biological materials to evaporate and reach the receiving substrate in droplet form. Following cell transfer from the ribbon, the substrate which contains a biopolymer or cell culture medium to maintain cellular adhesion and sustained growth. To print hydrogels, cells, proteins, and ceramic materials, LAB primarily employs nanosecond lasers with UV or near-UV wavelengths as energy sources. The resolution varies from pico- to micro-

scale features and is influenced by a number of factors, including the thickness of the biological materials on the film, their rheological properties, the energy of the laser pulse, the wettability of the substrate, and others. (Catros *et al.,* 2011) [2] Bioprinting Stem cells, including embryonic stem cells (ESCs), BMSCs, and ASCs, can be printed and patterned using a laser-assisted bioprinter through precise deposition of picoliter (pl) volumes of fluid or laser-aided accurate localization. An important concern in stem cell printing is that stem cell activity, including proliferation and pluripotency, may have the chance of change during the printing process.

Applications

Tissue engineering and regenerative medicine

With donor numbers declining there is a need for tissue engineering and regenerative medicine to fill the void. Several tissues have already been explored for 3D bioprinting, including the heart valve, myocardial tissue, blood vessels, and musculo-skeletal tissues. For these tissues to be successful they have to provide the proper environment to allow cell proliferation and differentiation. Their elasticity, flexibility and recovery rate need to mimic that of the native tissue environment. Hard tissue engineering mostly focusses on creating bone and cartilage tissues for aging diseases and the musculoskeletal system. Scaffolds, which mostly consist of polymers, ceramics and hydrogels, are commonly used to allow tissue regeneration. A great challenge in tissue engineering lies in the fabrication of cardiac tissues, due to the hierarchical structure of the myocardium and the need for angiogenesis.

Transplantation and clinical applications

Tissue engineering has been able to create multiple tissue types as *in vitro* models and for regenerative medicine applications. With the severe problem of tissue rejection after transplantation, 3D bioprinting can assist by creating personalized tissues. For personalized tissue transplants, 3D bioprinted structures need to resemble native tissues perfectly. DBB is a technique that can be used for the deposition of the bio-ink directly into the wound during surgery as it does not require contact and does not bear toxic or unsafe interventions.

Cancer research

The ability of 3D bioprinting to mimic the native environment can also be used in the study of cancer pathogenesis and metastasis. The DBB technology can fabricate high-resolution tissue models with great repeatability.

Customised 3D printed Dog Prosthetic

Prosthetic limbs are an emerging technology for the limb loosed animals. Dogs all over the world require amputations and many of these animals never have the chance to regain function of their limb due to cost and time to manufacture. Emerging technology are quicker for the production of 3D printed prosthetic. Improving animal prosthetics For a hind region leg of a dog, articulating knee joint is a unique and innovative step. Based on dog's anatomy the prosthetic could be altered by scaling and shortening / lengthening components to fit individual and residual limb. Additionally, owners should consult any Veterinarian before applying a 3D printed prosthetic on their dog because an ill-fitting or poorly designed prosthetic could worsen some medical conditions of dog's health. (Susan *et al.,* 2017) [23].

3D printed surgical models

3D models for veterinarians surgeons to physically hold and examine the skulls and bones. For veterinarians, the models can be printed and help students and veterinarians practise surgical procedures in advance of an operation by converting CAT scan findings into a format that 3D printers can recognise. The scientists are even experimenting with full-color models, which should allow them to test new approaches that avoid contact with vital blood vessels and other tissues.

Printing Living Skin with Blood Vessels

Currently, for patients in need of skin grafts, artificial skin products are required to replace the old method. In an autologous skin grafts, the doctors shave off a piece of healthy skin to cover the damaged area and create a new wound. Generally the artificial skin grafts are made from materials from bovine collagen to polymer foam. Artificial skin products have a range of limitation mainly they are often temporary, and don't resemble natural skin. (Khatiwala *et al.,* 2012) [11]. (Baillie K. 2015) [25].

Prosthetics and Orthotics

Much like with humans, prosthetics and orthotics are becoming increasingly popular in the animal world, especially with the innovations in veterinary medicine and research. The first 3D prosthetic was for a dog named Derby, from South Carolina in 2014. He suffered from no front paws. This bioprinting technology especially by 3D printing, the process was quick, cheap, and can be adjusted at any time with the flexibility and speed of additive manufacturing. (Melisa Gonzalez 2019) [24] (Quinn-Gorham and DM, Khan JM. 2016) [26].

3D printing technology

3D printer technology is providing veterinary medicine with a powerful tool to facilitate surgical planning, enhance the teaching of students, promote research, and improve client communication. The use of rapid prototyping is expected to increase as volumetric acquisition in MRI and 4D ultrasound. Finally, as technology evolves, more materials, including tissues, should be printable as suggested by current research on the printing of transplantable organs. The use of 3D printers is currently reported as being common in human radiology departments and has been integrated into the clinical routine. Radiologists have the anatomical and technical knowledge to use the Devices and Correct Any Condition. (Hespel *et al.,* 2014) [27].

Conclusion

Bioprinting has gained considerable success in many medical fields, mainly in veterinary science including surgery for dogs. 3D printed models can help to figure out anatomical details for the targeted small animals that 2D imaging and 3D virtual models wouldn't let you see. However, it will need additional manufacturing costs and time to access a 3D printer. However, light should be thrown over these concerns for a better outlook in the near future.

References

1. Boland T, Xu T, Damon B, Cui X. Application of inkjet printing to tissue engineering. Biotechnol J 2006;1:910-7.
2. Catros S, Fricain JC, Guillotin B, Pippenger B, Bareille R, Remy M, *et al*. Laser-assisted bioprinting for creating

on-demand patterns of human osteoprogenitor cells and nano-hydroxyapatite. Biofabrication 2011;3:025001.
3. Colin Jamieson, Patrick Keenan, D'Arcy Kirkwood, Saba Oji, Caroline Webster, Keith Russell A. *et al*. A Review of Recent Advances in 3D Bioprinting With an Eye on Future Regenerative Therapies in Veterinary Medicine. Front. Vet. Sci 2021;(**7**)16.
4. Cui X, Boland T, D'Lima DD, Lotz MK. Thermal inkjet printing in tissue engineering and regenerative medicine. Recent Pat Drug Deliv Formul. 2012;6:149-55.
5. Cui X, Dean D, Ruggeri ZM, Boland T. Cell damage evaluation of thermal inkjet printed Chinese hamster ovary cells. Biotechnol Bioeng 2010;106:963-9.
6. Derby B. Printing and prototyping of tissues and scaffolds. Science 2012;338:921-6.
7. Garreta E, Oria R, Tarantino C, Pla-Roca M, Prado P, Fernández-Avilés F. Tissue engineering by decellularization and 3D bioprinting. Mater Today 2017;20:166-78.
8. Ilze Donderwinkel, Jan van Hest CM, Neil Cameron R. Bio-inks for 3D bioprinting: recent advances and future prospects. Polym. Chem 2017;8:4451
9. Jipeng Li, Mingjiao Chen, Xianqun Fan, Huifang Zhou. Recent advances in bioprinting techniques: approaches, applications and future prospects. J Transl Med 2016;14:271.
10. JunJie Yu, Su A Park, Wan Doo Kim, Taeho Ha, Yuan-Zhu Xin, JunHee Lee *et al*. Current Advances in 3D Bioprinting Technology and Its Applications for Tissue Engineering. Polymers 2020;12:2958.
11. Khatiwala C, Law R, Shepherd B, Dorfman S, Csete M. 3D cell bioprinting for regenerative medicine research and therapies. Gene Ther 2012;**7**:1-19.
12. Lou T, Wang X, Song G, Gu Z, Yang Z. Structure and properties of PLLA/ beta- TCP nanocomposite scaffolds for bone tissue engineering. J Mater Sci Mater Med 2015;26:5366.
13. Matsiko A, Gleeson JP, O'Brien FJ. Scaffold mean pore size influences mesenchymal stem cell chondrogenic differentiation and matrix deposition. Tissue Eng Part A 2015;21:486-97.
14. Mohsen Askari, Moqaddaseh Afzali Naniz, Monireh Kouhi, Azadeh Saberi, Ali Zolfaghariane, Mahdi Bodaghi. Recent progress in extrusion 3D bioprinting of hydrogel biomaterials for tissue regeneration: a comprehensive review with focus on advanced fabrication techniques. Biomater. Sci 2021;9:535.
15. Murphy, Atala A. 3D bioprinting of tissues and organs. Nat Biotechnol 2014;32:773-85.
16. Nadeem D, Smith CA, Dalby MJ, Meek RM, Lin S, Li G *et al*. Threedimensional CaP/gelatin lattice scaffolds with integrated osteoinductive surface topographies for bone tissue engineering. Biofabrication 2015;7:015005.
17. Nakamura M, Kobayashi A, Takagi F, Watanabe A, Hiruma Y, Ohuchi K, *et al*. Biocompatible inkjet printing technique for designed seeding of individual living cells. Tissue Eng 2005;11:1658-66.
18. Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. Tissue Eng Part B Rev 2008;14:149-65.
19. Ozbolat IT, Hospodiuk M. Current advances and future perspectives in extrusion- based bioprinting. Biomaterials 2016;76:321-343.
20. Park SM, Yang S, Rye SM, Choi SW. Effect of pulsatile flow perfusion on decellularization. Biomed Eng Online 2018;17:15.
21. Pati F, Jang J, Lee JW. Extrusion bioprinting. Biomaterials 2015, 214-218.
22. Patrick Rider, Željka Perić Kačarević, Sujith Retnasingh, Said Alkildani, Ole Jung, Mike Barbeck. Bioprinting of tissue engineering scaffolds. Biomaterials 2018, 0251-0253.
23. Susan Aikman, James McGee. Ally Meile, Brandon Powel, Jessica Godard and Thomas Bitter. Prosthetics for Dogs and Other Pets. Biomaterials 3d HUBS, 2017, 1758-1763.
24. Melisa Gonzalez. 3D Printing Helps to Plan Limb Surgery on a Dog. Vet prac news 2019, 9-12.iuj8jiui9i89999999909
25. Baillie K. 3D Printing for Veterinary Surgeries. J Vet Sci Technol 2015:7:325-330
26. Quinn-Gorham, Khan JM. Thinking Outside of the Box: The Potential of 3D Printing in Veterinary Medicine. J Vet Sci Technol 2016;7:360.
27. Hespel A.-M, Wilhite R, Hudson J. Invited review-applications for 3d printers in veterinary medicine. Vet Radiology & Ultrasound 2014;55(4):347-358.