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Applications of 3D Printed Biomaterials in Reconstructive Surgery of the Anterior Skull Base

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Abstract: *Anterior skull base reconstruction is a complex surgical procedure that requires careful evaluation of the patient's condition and the expertise of a skilled surgical team. Skull base reconstruction objectives focus on providing water-tight separation between the intracranial and extracranial contents, closing dead space, and returning reasonable form and function. Regarding the reconstruction options, the main categories are non-vascular grafts, loco-regional flaps, free tissue transfer or bony-free flaps. The major challenge in reconstructive surgery is the effective sealing of the defect due to its uneven edges and the conformation of the anterior skull base. Given this challenge, we are considering the possibility of designing a prosthetic for anterior skull base defects using the 3D printer.*

Keywords: *anterior skull base, reconstructive surgery, 3D printer*

1. Introduction

The skull base is a three-dimensional structure that requires very good knowledge and understanding of the local anatomy for its surgical approach. The boundaries of the anterior skull base are medially, the crista galli and cribriform plate; laterally, the frontal bone; and posteriorly, the lesser wings of the sphenoid bone [1].

The two main categories of factors that can cause defects in the anterior skull base are traumatic and non-traumatic. Benign tumours like angiofibroma, inverted papilloma, osteomalacia and secondary mucocele or malignant tumours like sinonasal carcinoma, rhabdomyosarcoma or lymphoma represent the non-traumatic factors. The traumatic factors are defined by craniofacial trauma or surgical trauma. The main complication of skull base defects is cerebrospinal fluid (CSF) leaks [2]. With the development of technology, the endoscopic endonasal approach has become a significant option for the surgical treatment of anterior skull base pathologies and the reconstruction of anterior skull base defects [3]. A successful reconstruction of these defects represents the absence of postoperative CSF leaks, meningitis or pneumocephalus.

When planning skull base reconstruction surgery, one must consider the defect's location and size to assess the pressure of the CSF leak and the integrity of the dura. It is also essential to know if the history of the patient includes radiation therapy, meningitis or other skull base surgeries [4].

Regarding the reconstruction options, the main categories are non-vascular grafts, loco-regional flaps, free tissue transfer or bony-free flaps [5].

The major challenge in reconstructive surgery is the effective sealing of the defect due to its uneven edges and the conformation of the anterior skull base [6]. Given this challenge, we are considering the possibility of designing a prosthetic for anterior skull base defects using the 3D printer. When printing a 3D object, one must use computer-aided design (CAD) software. The fabrication of 3D-printed objects

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begins from the base, layer by layer, to the top. CAD guides the dispersion of each layer. 3D printing includes multiple techniques for creating objects with different material requirements, efficacy and costs [7-9].

Stereolithography (SLA) was the first 3D printing process developed and is still its gold standard to this day. It uses a moving CAD-controlled UV source to expose liquid resins to UV light. The exposure to UV radiation drives the resin into the solid phase. The leftover resin must be removed before final UV-chamber curing. The disadvantages of SLA are that it is a slow and expensive process [10,11].

Material jetting printing (MJP) uses an immobile UV source, unlike SLA. This makes the positional deposition of liquid resin essential in this process. There are two main types of jetting used by MJP machines: continuous and drop-on-demand. One of the main advantages of MJP is compositional control, which is achieved by dispensing individual drops of resin, which gives the possibility to adjust materials during the printing process [12,13].

Binder jetting printing (BJP) uses a powder base and a binder substance. After drying the powder base and the binder, one can add glass, sand or metals. After the fabrication of all the layers, substantial processing is required, and the object needs depowdering and sintering to improve its mechanical properties. Afterwards, additional materials are infiltrated and annealed for better structural integrity. This technique's main disadvantage is its inferior resolution capabilities [14,15].

Selective laser sintering (SLS) technique is based on altering deposited powder. A laser is used to sinter and melt the powder. The materials used by SLS include nylon, metal, resin, polymers, and ceramic. The ability to produce soft scaffolding, which is conductive for soft tissue, represents one of the main advantages of SLS [16].

Fused deposition modelling (FDM), unlike BJP or SLS, does not need to interact with a powder or binding substance because the material can be injected directly onto the fabrication platform. Each layer is printed by a platform that moves vertically. The production of each layer has heating of the material as the first step until it reaches a semi-molten state. Afterwards, the material is extruded through nozzles, where it solidifies. FDM can use materials such as metals, ceramics and thermoplastic substances that must be pliable enough to be extruded. The technique's disadvantages are poor resolution and poor surface finish [16].

This paper aims to evaluate the existing 3D-printed biomaterials in order to find the most suitable one for producing an implant that could be used to seal anterior skull base defects.

2. Materials and methods

The biomaterials must meet specific requirements: they must be biomimetic and biocompatible, not to form toxic degradation byproducts, they should be easily manufactured, structurally stable and affordable.

Polymers are the most used biomaterials in 3D printing technologies. They come in various forms that can each be used in a different printing technique. For example, filaments are used for FDM, powder beads are used for SLS, solutions are used for SLA, and gels are used for direct ink writing (DIW).

Polymers are biocompatible and can be dissolved in organic solvents [17].

Acrylonitrile butadiene styrene (ABS) is a petrochemical triblock copolymer and was one of the first 3D printing materials used. It is flexible, not biodegradable, shrinks in contact with air, can resist temperatures of -20°C to 80°C, and has a melting point of 105°C. This material can be used for fused deposition modelling and stereolithography systems [18, 19].

Polylactic acid is the main polymer used for fused deposition modelling. The main advantages are its low cost and the fact that it can be produced by using cornstarch. The main disadvantage of this polymer is the release of lactic acid byproducts when it degrades. These byproducts can cause inflammation or cell death. A solution for this problem is combining it with carbonated calcium phosphates that neutralize acidity. Polylactic acid has good compressive strength compared to bone tissue; thus, it is usually used in orthopaedic support [20,21].

Polycaprolactone (PCL) is also a low-cost and biodegradable polyester. It melts at 60°C, and it has superior viscoelastic properties. It can be used for FDM printing to produce long-term degradable implants. An important aspect is that PCL is stable in the body for around six months and completely degrades in three years without forming harmful byproducts.

PCL can produce scaffolds with SLS and DIW because it forms interconnected micro-fibres that lead to a scaffold with porosity suited for embedding growth factors, drugs and cells [22, 23].

High-performance polymers - polyether ether ketone (PEEK), polyether ketone ketone (PEKK), polyetherimide (Ultem).

These high-performance polymers have mechanical characteristics similar to those of metals, like high mechanical and thermal resistance and good strength. The main difference is that these polymers are much lighter than some metals. These properties make them attractive for the biomedical field.

PEEK, PEKK, and Ultem have a melting point of ~ 350°C. For this reason, they cannot be printed on all FDM printers. The 3D printer must meet specific requirements to print these polymers: it needs to have a heating plate capable of reaching at least 230°C, an extrusion at 350°C, and a closed chamber. This property gives 3D-printed high-performance polymers superior heat resistance, meaning they can undergo steam sterilization without softening [24].

PEEK has osseointegration properties, is biocompatible, has low moisture absorption, and is radiolucent. These properties make PEEK a good option for bone tissue engineering, such as craniofacial implants [25-27].

Polypropylene (PP)

PP is a crystalline thermoplastic polymer. This polymer is produced from propene (or propylene) monomer. The melting point of PP is ~ 165°C. PP has a density of 0.908 g/cm^3, making it one of the lightest polymers. PP is known for being a strong material that resists well to abrasion and has the ability to absorb shocks.

Its disadvantages include low-temperature resistance (PP becomes fragile at -20°C), it expands under UV rays, and chlorinated solvents can cause it to swell.

PP properties make it useful in producing personalized 3D-printed orthoses for patients with bone fractures [28].

Polyamides

Polyamides are usually used with SLS technology. However, nylon, a variant of polyamides, is also available for FDM.

Polyamides are biocompatible and are usually used to produce scaffolds or prostheses that come into contact with skin.

Polyamides also have good stability, rigidity, flexibility, and shock resistance. In the medical field, polyamides are combined with hydroxyapatite to produce porous scaffolds used for bone regeneration. [29-31].

Thermoplastic polyurethane (TPU)

Although TPU is flexible and abrasion-resistant, it cannot withstand high temperatures [32]. Its properties make TPU the ideal option for insoles, foot orthoses or thumb supports [33].

Polymeric hydrogel-based inks

Hydrogels are 3D cross-linked polymer networks that can retain large amounts of water (> 90%) [34, 35].

Due to hydrogels biocompatibility, tunable mechanics, and degradation, they are able to mimic extracellular matrix microenvironments (ECM) [36, 37].

Their ability to incorporate nucleic acids, glycans, growth factors or fatty acids leads to the possibility of creating biomimetic supramolecular scaffolds.

The properties of hydrogel make it a valuable tool in the biomedical field (like regenerative medicine) but also in the engineering one.

Bio-inks are how printable hydrogels are named when they contain biochemical molecules. Bio-inks are defined by Groll et al.as "a formulation of cells suitable for processing by an automated bio-fabrication technology that may also contain biologically active components and biomaterials" [38].

The most used printing systems for bioinks are inkjet, light-assisted, and extrusion-based 3D printing [39-41].

The main requirements that hydrogel inks must meet to be bioprinted are to gellify rapidly, flow under modest pressure, and have good mechanical properties. Because bioinks' properties can affect cell growth, when choosing bioinks, one should consider the physiological aspects and mechanical properties of the desired tissue. In recent years, many hydrogels have been developed. Still, the most used ones for 3D bioprinting are those made from natural or synthetic proteins - for example, collagen, gelatin, spider silk, ECM-derived proteins, and polysaccharides.

Ceramic-based materials could potentially be helpful for dental surgery and orthopaedics due to their high stiffness (393 GPa). Until now, 3D printing methods have been limited in ceramic printing because of their high melting temperature, which is above 2000°C.

3. Results and discussions

Developing 3D printing techniques for head and neck reconstructive surgeries is a fascinating prospect. Nowadays, applications for 3D printing in otolaryngology are just in the preliminary stages of development. Extended skull base approaches are intricate and lengthy operations whose main challenge is closing the skull base defect created at the time of surgery. Although reconstructive techniques have become more successful over recent years, we believe that creating models and prostheses that cover the defects using the 3D printer could reduce operating time and wound exposure and improve closure effectiveness.

The main challenge is that skull base defects may vary depending on the location of the tumour and the amount of bone that needs to be removed for access. The ideal scenario is to be able to perform an intraoperative CT once the bony defect is completed and to print a prosthesis that fits perfectly the size of the opening. Unfortunately, currently, the 3D printing and modelling speeds are time-consuming and exceed the time frame of the surgery. Another problem is the sterilization of the 3D model after printing. The material used to produce 3D models should resist fast sterilization techniques to speed up the sterilization process.

Another option is preparing the 3D model before the surgery, relying on selecting the anatomical landmarks on the preoperative CT scans. The preselected landmarks should be sufficiently precise to guide the surgeon in tailoring the model prosthesis. CT scans must be performed in bone window with thin cut slices.

The elasticity of the prosthetic model is an essential aspect that needs to be considered. Prostheses with a high rigidity can be difficult to insert into the nasal cavity and can cause damage to the mucosa and surrounding tissues. Using flexible materials can reduce the risk of mucosal injury and facilitate the insertion of the 3D-printed model into the nasal cavity. On the other hand, if the flexibility is too high, the model won't be able to hold its shape and place after insertion. For these reasons, it is necessary to find a balance between flexibility and rigidity of the 3D printed model so that it is easy to insert at the level of the nasal cavity and the skull base defect but rigid enough to hold its place and have good stability.

The 3D-printed products used for medical purposes must be biocompatible, not to trigger allergic or toxic reactions.

In addition, researchers are continuously exploring the use of 3D printing for multiple medical applications, including producing tailored drug delivery devices fabricating tissues and organs for regenerative medicine. 3D printing technology is also used to understand the complex structural-

physiology relationships regarding diagnosing and treating pulmonary, neurological, or cardiovascular diseases [42-44].

In selecting the biomaterial, we took into consideration its application and functionality.

We compared the materials' functionality by dividing them into three main categories: strength, special properties and flexibility. The special properties are subdivided into the following categories: chemical resistance, biodegradable, biocompatible, sterilizable and bioactive. The flexibility category is subdivided into high elongation and rubber-like.

ABS has low strength (less than 40MPa), is sterilizable and is best suited for surgical models and prostheses.

TPU is biodegradable and has a high flexibility.

PEEK and PEKK have high strength (more than 85 MPa) and chemical resistance, are biocompatible, and are sterilizable. These properties make them suitable for creating load-bearing implants and surgical tools.

Other advantages of PEEK are osseointegration, low moisture absorption, and the fact that it is radiolucent. These properties make PEEK a good option for bone tissue engineering, such as craniofacial implants.

After thoroughly analyzing the properties of the 3D-printing biomaterials, we concluded that PEEK is the biomaterial that best defines our project purpose.

4. Conclusions

Endoscopic skull base surgery is increasingly indicated in a variety of pathologies, and the tendency is for it to become the standard of care. Anterior skull base surgery presents ablative and reconstructive challenges for surgeons. After these surgeries, there is a subsequent risk of postoperative CSF leaks. Currently, multilayer reconstruction techniques are used to seal the defects with a high success rate. We believe these results can be improved by creating a 3D-printed model of the skull base defect.

As technology advances, new biocompatible materials will appear, and the time required for printing and tailoring the prosthesis will be reduced. The quality and applicability of this approach will be improved. Additional laboratory and clinical investigations are necessary to demonstrate the relevance and efficiency of the technique.

References

1. KONG, D.S., KIM, H.Y., KIM, S.H., MIN, J.Y., NAM, D.H., PARK, K. et al., Challenging reconstructive techniques for skull base defect following endoscopic endonasal approaches. *Acta Neurochir* (Wien) 153:807–813, 2011
2. BEDROSIAN, J.C., ANAND, V.K., SCHWARTZ, T.H., The endoscopic endonasal approach to repair of iatrogenic and noniatrogenic cerebrospinal fluid leaks and encephaloceles of the anterior cranial fossa. *World Neurosurg* 82 (6 Suppl):S86–S94, 2014
3. HARVEY, R.J., PARMAR, P., SACKS, R., ZANATION, A.M., Endoscopic skull base reconstruction of large dural defects: a systematic review of published evidence. *Laryngoscope* 122:452–459, 2012
4. HEGAZY, H.M., CARRAU, R.L., SNYDERMAN, C.H., KASSAM, A., ZWEIG, J., Transnasal endoscopic repair of cerebrospinal fluid rhinorrhea: a meta-analysis. *Laryngoscope*. 2000;110(7):1166–1172
5. REYES, C., MASON, E., SOLARES, C.A., Panorama of reconstruction of skull base defects: from traditional open to endonasal endoscopic approaches, from free grafts to microvascular flaps. *Int Arch Otorhinolaryngol*. 2014 Oct. 18 (Suppl 2):S179-86
6. LEE, S.C., SENIOR, B.A., Endoscopic skull base surgery. *Clin Exp Otorhinolaryngol*. 2008 Jun. 1(2):53-62.
7. FAHMY, M.D., JAZAYERI, H.E., RAZAVI, M., MASRI, R., TAYEBI, L., Three-dimensional bioprinting materials with potential application in preprosthetic surgery. *J Prosthodont* 25:310–318, 2016

8. GROSS, B.C., ERKAL, J.L., LOCKWOOD, S.Y., CHEN, C., SPENCE, D.M., Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences. *Anal Chem*. 2014;86(7):3240-3253.
9. PARTHASARATHY, J., 3D modeling, custom implants and its future perspectives in craniofacial surgery. *Ann Maxillofac Surg*. 2014;4(1):9-18.
10. SKOOG, S.A., GOERING, P.L., NARAYAN, R.J., Stereolithography in tissue engineering. *J Mater Sci Mater Med*. 2014;25(3):845-856.
11. MARRO, A., BANDUKWALA, T., MAK, W., Three-dimensional printing and medical imaging: a review of the methods and applications. *Curr Probl Diagn Radiol*. 2016;45:2-9.
12.TUMBLESTON, J.R., SHIRVANYANTS, D., ERMOSHKIN, N., et al. Additive manufacturing: continuous liquid interface production of 3D objects. *Science*. 2015;347:1349-1352.
13.STUDART, A.R., Additive manufacturing of biologically-inspired materials. *Chem Soc Rev*. 2016;45:359-376.
14. METEYER, S., XU, X., PERRY, N., ZHAO, Y., Energy and material flow analysis of binder-jetting additive manufacturing processes. *Procedia CIRP*. 2014(15):19-25.
15. ONO, I., ABE, K., SHIOTANI, S., HIRAYAMA, Y., Producing a full-scale model from computed tomographic data with the rapid prototyping technique using the binder jet method: a comparison with the laser lithography method using a dry skull. *J Craniofac Surg*. 2000;11:527-537.
16. CHIA, H.N., WU, B.M., Recent advances in 3D printing of biomaterials. *J Biol Eng*. 2015;9:4.
17. LIGON, S.C., LISKA, R., STAMPFL, J., GURR, M., MULHAUPT, R., Polymers for 3D printing and customized additive manufacturing. *Chem Rev*, 117 (15) (2017), pp. 10212-10290
18. CASAVOLA, C., CAZZATO, A., MORAMARCO, V., RENNA, G., Mechanical behaviour of ABS-fused filament fabrication compounds under impact tensile loadings *Materials* (Basel), 12 (8) (2019)
19. MOHAMED, O.A., MASOOD, S.H., BHOWMIK, J.L., Analytical modelling and optimization of the temperature-dependent dynamic mechanical properties of fused deposition fabricated parts made of PC-ABS. *Materials* (Basel), 9 (11) (2016)
20. VALERGA, A.P., BATISTA, M., FERNANDEZ-VIDAL, S.R., GAMEZ, A.J., Impact of chemical post-processing in fused deposition modelling (FDM) on Polylactic Acid (PLA) surface quality and structure. *Polymers* (Basel), 11 (3) (2019)
21. KOH, J.J., ZHANG, X., HE, C., Fully biodegradable Poly(lactic acid)/Starch blends: a review of toughening strategies. *Int J Biol Macromol*, 109 (2018), pp. 99-113
22. Woodruff, M.A., Hutmacher, D.W., The return of a forgotten polymer-Polycaprolactone in the 21st century. *Prog Polym Sci*, 35 (10) (2010)
23. ESHRAGHI, S., DAS, S., Mechanical and microstructural properties of polycaprolactone scaffolds with one-dimensional, two-dimensional, and three-dimensional orthogonally oriented porous architectures produced by selective laser sintering. *Acta Biomater*, 6 (7) (2010), pp. 2467-2476
24.KURTZ, S.M. Chemical and radiation stability of PEEK. *Peek Biomaterials Handbook* (2012)
25.KANG, J., ZHANG, J., ZHENG, J., WANG, L., LI, D., LIU, S., 3D-printed PEEK implant for mandibular defects repair - a new method. *J Mech Behav Biomed Mater*, 116 (2021), Article 104335
26.GU, X., SUN, X., SUN, Y., WANG, J., LIU, Y., YU, K., WANG, Y., ZHOU, Y., Bioinspired modifications of PEEK implants for bone tissue engineering. *Front Bioeng Biotechnol*, 8 (2020), Article 631616
27. HO, D., PHAN, T.H.T., MOBBS, R.J., REDDY, R., The use of radiolucent (carbon fibre-reinforced polymer) pedicle screw fixation for serial monitoring of clear cell meningioma: a case report. *J Surg Case Rep*, 2020 (12) (2020), p. rjaa510
28. LIN, H., SHI, L., WANG, D., A rapid and intelligent designing technique for patient-specific and 3D-printed orthopedic cast. *3D Print Med*, 2 (1) (2015), p. 4
29.WANG, H., LI, Y., ZUO, Y., LI, J., MA, S., CHENG, L., Biocompatibility and osteogenesis of biomimetic nano-hydroxyapatite/polyamide composite scaffolds for bone tissue engineering. *Biomaterials*, 28 (22) (2007), pp. 3338-3348

30.KHADKA, A., LI, J., LI, Y., GAO, Y., ZUO, Y., MA, Y., Evaluation of hybrid porous biomimetic nano-hydroxyapatite/polyamide 6 and bone marrow-derived stem cell construct in repair of calvarial critical size defect. *J Craniofac Surg*, 22 (5) (2011), pp. 1852-1858
31.LI, J., LI, Y., MA, S., GAO, Y., ZUO, Y., HU, J., Enhancement of bone formation by BMP-7 transduced MSCs on biomimetic nano-hydroxyapatite/polyamide composite scaffolds in repair of mandibular defects. *J Biomed Mater Res A*, 95 (4) (2010), pp. 973-981
32.FENG, F., YE, L., Morphologies and mechanical properties of polylactide/thermoplastic polyurethane elastomer blends. *J Appl Polym Sci*, 119 (5) (2010)
33.VAN ALSENOY, K., RYU, J.H., GIRARD, O., The effect of EVA and TPU custom foot orthoses on running economy, running mechanics, and comfort. *Front Sports Act Living*, 1 (2019), p. 34
34. ZHANG, Y.S., KHADEMHOSSEINI, A., Advances in engineering hydrogels. *Science*, 356 (6337) (2017)
35.PEPPAS, N.A., HILT, J.Z., KHADEMHOSSEINI, A., LANGER, R., Hydrogels in biology and medicine: from molecular principles to Bionanotechnology. *Adv Mater*, 18 (11) (2006)
36.GECKIL, H., XU, F., ZHANG, X., MOON, S., DEMIRCI, U., Engineering hydrogels as extracellular matrix mimics. *Nanomedicine* (Lond), 5 (3) (2010), pp. 469-484
37.TIBBITT, M.W., ANSETH, K.S., Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol Bioeng*, 103 (4) (2009), pp. 655-663
38.GROLL, J., BURDICK, J.A., CHO, D.W., DERBY, B., GELINSKY, M., HEILSHORN, S.C., JUNGST, T., MALDA, J., MIRONOV, V.A., NAKAYAMA, K., OVSIANIKOV, A., SUN, W., TAKEUCHI, S., YOO, J.J., WOODFIELD, T.B.F., A definition of bioinks and their distinction from biomaterial inks. *Biofabrication*, 11 (1) (2018), Article 013001
39.PLACONE, J.K., ENGLER, A.J., Recent advances in extrusion-based 3D printing for biomedical applications. *Adv Healthc Mater*, 7 (8) (2018), Article e1701161
40.WU, C., WANG, B., ZHANG, C., WYSK, R.A., CHEN, Y.W., Bioprinting: an assessment based on manufacturing readiness levels. *Crit Rev Biotechnol*, 37 (3) (2017), pp. 333-354
41.KIRCHMAJER, D.M., GORKIN, R., PANHUIS, M.I.H., An overview of the suitability of hydrogel-forming polymers for extrusion-based 3D-printing. *J Mater Chem B*, 3 (20) (2015), pp. 4105-4117
42.MECA, A.D., TURCU-STIOLICA, A., BOGDAN, M., SUBTIRELU, M.S., COCOS, R., UNGUREANU, B.S., MAHLER, B., PISOSCHI, C.G., Screening performance of C-reactive protein for active pulmonary tuberculosis in HIV-positive patients: A systematic review with a meta-analysis. *Front. Immunol*., 13 (2022). https://doi.org/10.3389/fimmu.2022.891201.
43. MOTOC, N.S., MARTINOVICI, P., MAHLER BOCA, B., TUDORACHE, I.S., HARSOVESCU, T., FURTUNESCU, F.L., MAN, M.A., POP, C.M., Neutrophil-to-lymphocyte ratio (NLR) and Platelets-to-lymphocyte (PLR) Ratio in Patients with Exacerbation of Bronchiectasis, *Rev. Chim.*, 70(11), 2019, 3889-3892. https://doi.org/10.37358/RC.70.19.11.7665
44. TIU, V.E., POPESCU, B.O., ENACHE, I.I., TIU, C., TERECOASA, E., PANEA, C.A., Serum and CSF Biomarkers Predict Active Early Cognitive Decline Rather Than Established Cognitive Impairment at the Moment of RRMS Diagnosis. *Diagnostics*. 2022; 12(11):2571. https://doi.org/10.3390/diagnostics12112571

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