DOI: 10.24425/academiaPAS.2024.151176

Body Parts from Bioprinting

IM IMAGERY/SHUTTERSTOCK.COM

3D bioprinting not only represents the future of regenerative medicine, it can also facilitate more precise and more ethical scientific research.

Agata Kurzyk

Department of Cancer Biology
Maria Sklodowska-Curie
National Research Institute of Oncology,
Warsaw, Poland

In recent decades, the average human lifespan has lengthened. This global trend is certainly evident in Poland. According to 2022 data from Statistics Poland (GUS), women in Poland now live approximately six years longer than in 1990, while men live about seven years longer. Advances in cosmetology and aesthetic medicine have made it possible to maintain a youthful appearance for a longer time. Regrettably, this does not result in improved overall functioning of the human body, which typically declines with age. Unlike some other organisms, humans have a limited capacity for regeneration, and this capacity decreases with age. An aging population therefore entails higher healthcare costs. However, new advancements in the field of biomaterials can help address this issue. If harnessed in medicine, they will not only improve the quality of life but also potentially extend it.

Biomaterials

The use of biomaterials in medicine actually dates back to ancient times. Initially, various natural materials such as wood, gold, silver, and even animal bones were used to reconstruct or replace damaged body parts. In one of the oldest examples, dental implants were found in a skull at Faïd Souar in Algeria, dating back over 7,000 years. These implants were pieces of bone positioned to replace the second upper premolar.

The modern approach to regenerative medicine uses both natural materials, such as collagen, chitin, and gelatin, as well as synthetic materials, like polymers. Particularly noteworthy in this respect are biodegradable polymers, such as polylactide (PLA), polyglycolide (PGA), polylactide-glycolide copolymer (PLGA), and polycaprolactone (PCL), along with bioactive ceramics like tricalcium phosphate (TCP) and hydroxyapatite (HAP). Composites, which combine at least two different materials, also play a significant role. By merging various properties, they are becoming an attractive option in regenerative medicine and tissue engineering.

The biomaterials used in such applications must meet many requirements. The primary one is biocompatibility – to avoid rejection by the body, a biomaterial should not negatively affect tissues. Its structure is also crucial – porosity facilitates the growth of blood vessels and the transport of nutrients into the implant. Additionally, it is important for the material to bond easily with the patient's tissues and be biodegradable, allowing it to be replaced by the host's own cells.

Current techniques

Many techniques have been developed for creating implants to be used in the regeneration of damaged tissues, such as bones or skin. For bone implants, a structure is designed to function as a scaffold, supporting the cells necessary for reconstructing the damaged tissue. This scaffold can be made from biodegradable polycaprolactone (PCL), a material also used in dissolvable surgical sutures. This biomaterial can be seeded with cells that will eventually form bone tissue. Such cells can be obtained, for example, from adipose tissue acquired during routine liposuction procedures in aesthetic medicine clinics. The adipose tissue is processed using enzymes and multiple centrifugation steps. Ultimately, an isolated mixture of cells is obtained, which is then cultured *in vitro* and differentiated into bone-forming cells (osteoblasts). These prepared cells are then seeded onto the biomaterials, creating a biologically active bone implant that can replace the lost part of the body – although currently only small parts. Importantly, such an implant can be customized to the individual needs of the patient.

> Bioprinting make uses of a "bio-ink" – a hydrogel with jelly-like consistency that contains cells.

Another approach is to develop materials containing inorganic components that are actually present in human bone, such as calcium, magnesium, and

Agata Kurzyk, PhD
is a biotechnologist specializing in stem cell and cancer biology, as well as in biomedical engineering and the development of biomaterials for regenerative medicine. She employs 3D bioprinting technology to create advanced cell models, which facilitate studies of the tumor microenvironment, assessing drug effectiveness, and identifying treatment resistance mechanisms.
agata.kurzyk@nio.gov.pl

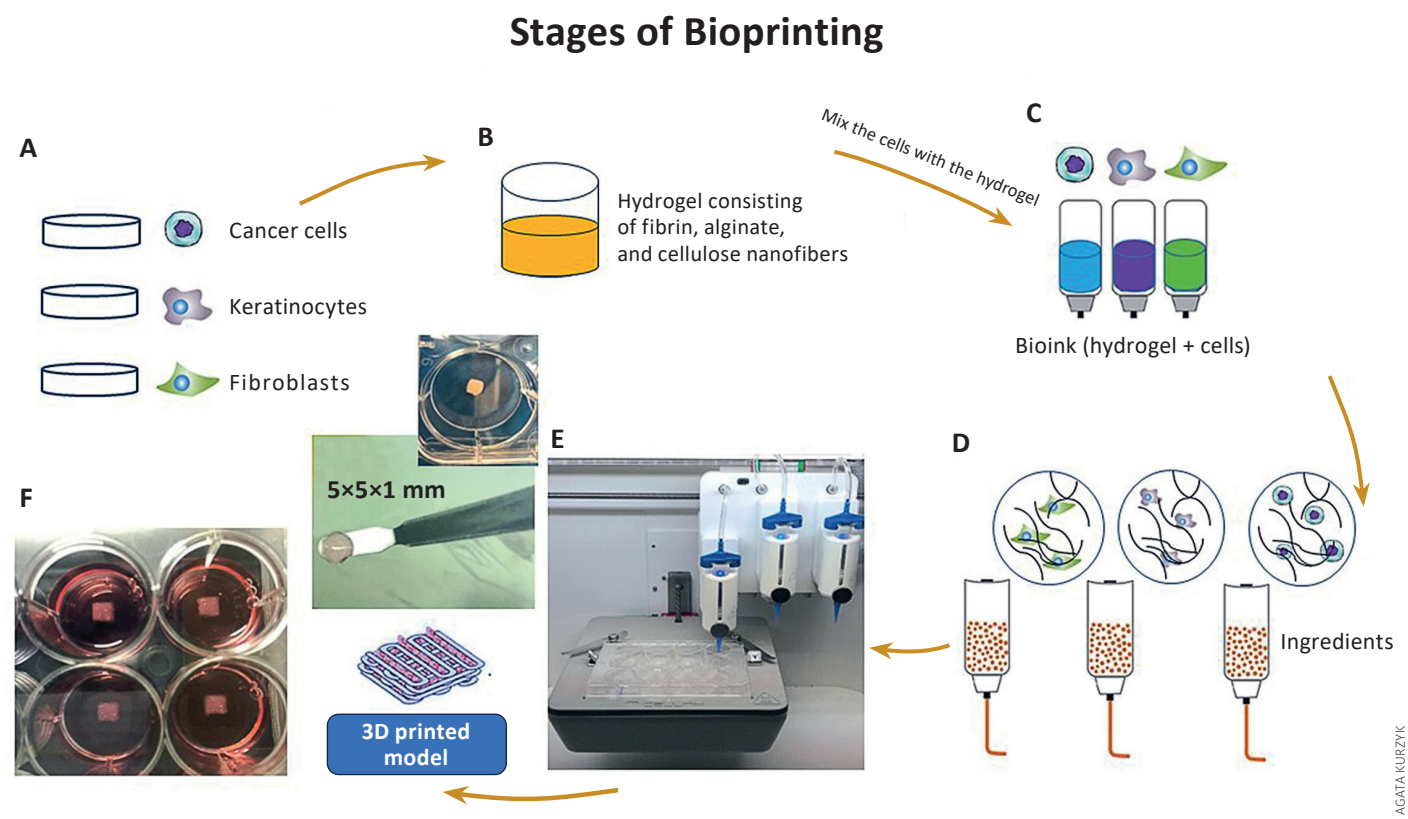
phosphorus compounds. These components give bone strength and resilience to injury. In the production of implants, nanohydroxyapatite is used instead of natural bone components. Nanohydroxyapatite is synthetically derived from calcium and phosphorus. By mixing these components appropriately, nanohydroxyapatite precipitates from the solution. The obtained material can then be modified through ion substitution, where some calcium ions may be replaced with magnesium, strontium, or zinc ions. The resulting biomaterial undergoes thermal treatment at 1200°C, improving its physicochemical and biological properties. Adding nanohydroxyapatite nanoparticles to implant materials enhances their biocompatibility, strengthens the implant structure, and improves its mechanical properties and integration with the surrounding tissue. This modified material supports regeneration processes by stimulating cells to proliferate and differentiate into osteoblasts. Furthermore, the released ions enhance the biological activity of biomaterials, stimulating bone formation (osteogenesis). These materials also possess anti-inflammatory and antibacterial properties, which contribute to faster healing and prevent infections.

Individual elements

The latest method for creating implants is 3D bioprinting technology. This concept was inspired by the operation of a regular printer. In bioprinting, instead of ink, a "bio-ink," which is a hydrogel with jelly-like consistency that contains cells, is used. The bio-ink is printed three-dimensionally, layer by layer, and then the resulting construct is cross-linked (hardened) to maintain its cohesive structure. Due to its precision and ability to control the structure, 3D bioprinting has found applications in many fields of biomedicine. One such application is the *in vitro* creation of new skin, which aims to replicate both the appearance and functions of natural tissue.

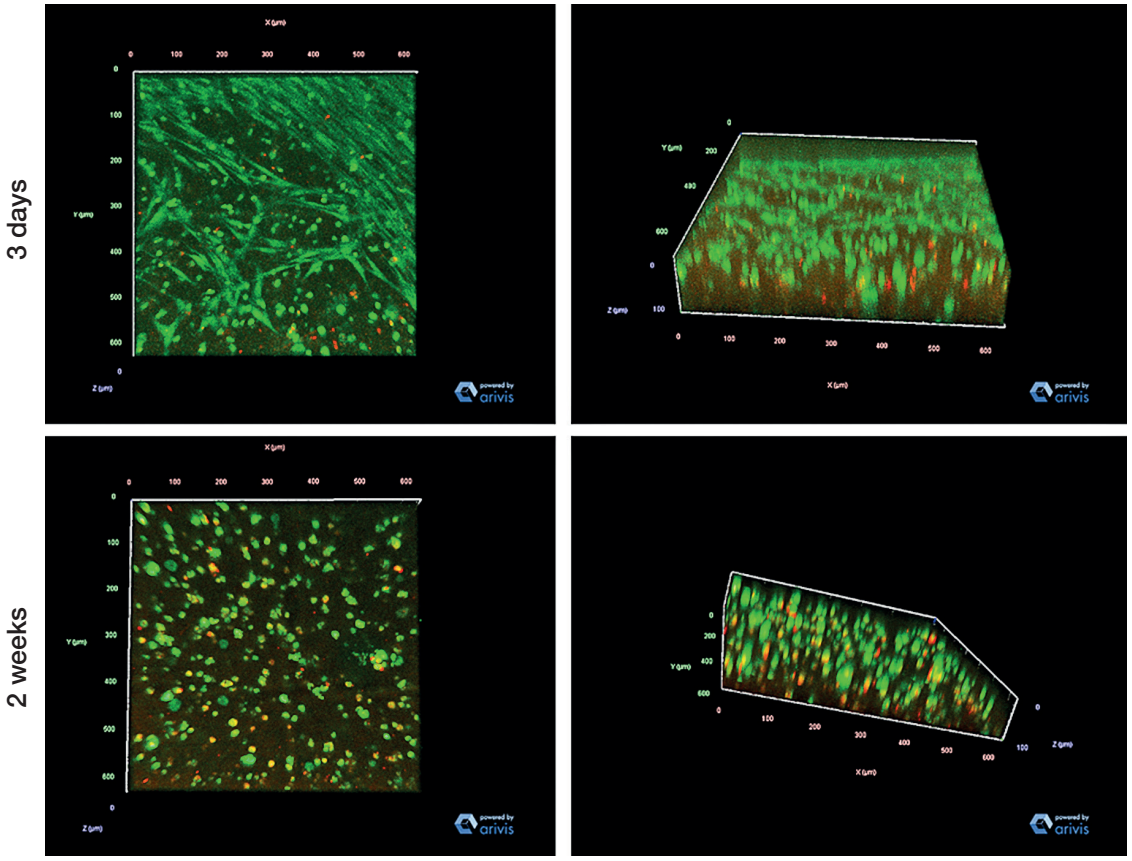
In natural skin tissue, the outer layer is the epidermis, primarily composed of dead skin cells, keratinocytes, and melanocytes, which produce melanin. The epidermis serves as the skin's protective barrier against external factors such as UV radiation. Below the epidermis lies the dermis, consisting of various cell types, including fibroblasts, which produce collagen, elastin, and other structural proteins, forming the extracellular matrix. The dermis provides the skin with elasticity and strength. The deepest layer of the skin is the subcutaneous layer, mainly composed of adipose tissue that serves as insulation and energy storage and contains larger blood vessels and nerves.

Printed skin also consists of various cell types forming distinct layers. During printing, the cells are deposited layer by layer and suspended in a hydrogel that mimics the extracellular matrix. Initially, this structure does not resemble natural tissue. However,



AGATA KURZYK

The bioprinting process:
A – cell cultures,
B – hydrogel preparation,
C – bio-ink preparation,
D, E – bioprinting and manufacturing of 3D models,
F – culture of printed 3D models



The location of cells in printed skin, after three days and two weeks of culture, initially reveals minor differences in cell shape. After several weeks, they acquire characteristic forms resembling fibroblasts (spindle-shaped) and keratinocytes (round). The different colors indicate live cells (green) and dead cells (red)

AGATA KURZYK

over several weeks of culture, it becomes more similar to natural tissue as a result of cell proliferation and migration of cells within the printed model. The application of printed tissues is becoming a promising approach not only for skin reconstruction after burn injuries but also for testing the safety and effectiveness of cosmetics, drugs, and chemicals.

Substitutes and 3D models

Thanks to new and advanced techniques in creating three-dimensional cellular structures, there are now endless possibilities for replicating various types of tissues. In addition to epithelial tissue, such research also encompasses muscle, nerve, and vascular tissue. These techniques not only aid in the reconstruction of damaged body parts but are also used in efforts to develop organ substitutes, crucial for improving the availability of transplants and better tailoring them to individual patient needs. Furthermore, these innovative approaches facilitate the study of cell aging processes, potentially leading to the development of more effective methods for delaying aging.

The use of new technologies that simulate tissue models provides an alternative to the use of animals in preclinical research, which is increasingly criticized due to ethical concerns. These three-dimensional models contribute to the advancement of more precise and efficient research methods, particularly in anticancer drug studies. Scientific evidence indicates that approximately 90% of promising therapies fail in clinical trials, primarily due to challenges in accurately replicating the human tumor microenvironment, comprising various cells such as fibroblasts, immune cells, and blood vessel cells alongside cancer cells. Multicellular three-dimensional bioprinted tumors provide a more accurate model of the cancerous tumor. Recent studies suggest that cancer cells in 3D models exhibit different responses to tested drugs compared to those in traditional cell cultures. Thus, the models facilitate a more precise evaluation of response to therapies and enable prediction of the development of drug resistance.

Undoubtedly, advancements in new biomaterials and 3D bioprinting technology are opening up exciting new horizons in medicine, potentially making treatments significantly more effective and enhancing our quality of life.

The author's research on developing an innovative model of the microenvironment of squamous cell carcinoma of the skin using three-dimensional bioprinting (3D) technology and evaluating the response to cetuximab is funded by the Count Jakub Potocki Foundation (grant No. 300/2021).

Further reading:

Kurzyk A., Szumera-Ciećkiewicz A., Miłoszewska J., Chechlińska M., 3D modeling of normal skin and cutaneous squamous cell carcinoma. A comparative study in 2D cultures, spheroids, and 3D bioprinted systems, *Biofabrication* 2024.

Kurzyk A., Szwed-Georgiou A., Pagacz J., Antosik A., Tymowicz-Grzyb P., Gerle A., Szterner P., Włodarczyk M., Płociński P., Urbaniak M.M., Rudnicka K., Biernat M., Calcination and ion substitution improve physicochemical and biological properties of nanohydroxyapatite for bone tissue engineering applications, *Scientific Reports* 2023.

Kurzyk A., Ostrowska B., Święszkowski W., Pojda Z., Characterization and Optimization of the Seeding Process of Adipose Stem Cells on the Polycaprolactone Scaffolds, *Stem Cells International* 2019.