The Method of Artificial Organs Fabrication Based on Reverse Engineering in Medicine

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Abstract The paper presents the concept and implementation of innovative methods of producing artificial organs and prosthesis based on 3D printing technology. These organs possess physical and mechanical properties similar to human organs and bodies part. As a result, using such organs, it is possible to conduct training and workshops, especially in the field of urological surgery, under the conditions close to real operations. Due to the fabrication of 3D models can also lead so-called pre-operations in order to better prepare surgeons to carry out complex operations and post-operation e.g. observers proper operation. The proposed method enables the production of artificial human organs whose consistency, plastic properties, hardness, elasticity are close to the real organ of specific patient, because it can be made on the basis of the data from MRI and CT. The process of preparing 3D geometry is prepared in applications in the field of CAD, but also through advanced applications designed for editing in vector geometry environment.

1 Introduction

Many advances in medicine have been achieved as a result of advances in other fields, including rehabilitation engineering, medical IT and robotics. This makes biomedicine open to novel approaches and technologies, which can increase effectivity of the therapeutic interventions. 3D printing, 3D scanning and associated

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technologies can constitute another step toward better application of the medicine and rehabilitation engineering principles in current clinical practice.

Current medical applications of 3D techniques cover many areas including:

- learning, including customized anatomical models, individual and team training in medical simulation centers,
- scientific research, concerning e.g. bones or soft tissues modeling and testing,
- 2D or 3D diagnostics (CT, MRI, 3D scans, clinical motion analysis, etc.)—2D images can be converted to 3D images,
- 3D scanning (including fast face/head scanning and fast whole body scanning),
- 3D printing in surgery, drug fabrication and rehabilitation engineering,
- reverse engineering (digitizing of real objects, partly for replication purposes),
- additive manufacturing, which allows for geometric flexibility of printed products (e.g. implants) and customization to suit individual needs.

Traditional manual design and manufacturing of personalized therapeutic solutions can be changed thanks to computer-aided design (CAD), rapid prototyping (RP), and computer-aided manufacturing (CAM) of physical models or even final products directly from 3D computer data. Joining various methods and techniques (e.g. within reveres engineering) allows to significantly shorten track between measurements in particular patient and ready-to-use personalized equipment. Expensive, high-end 3D technologies are currently replaced by much cheaper commercial technologies that have proven much cheaper, offering the same quality, geometric accuracy, and shape reconfiguration possibilities. Each 3D copy reflects the same original (or modified by therapists/manufacturer) data—there is lack of e.g. plastic mold or wrap destroyed irreversibly in the process of fabrication. Modification of geometrical parameters and material features of the model or final product is possible on almost every stage of the manufacturing. In selected cases, where more specialized knowledge and experience is required, rapid prototyping can be regarded only as quicker alternative for contemporary approaches. Another problem constitutes application of proper materials in particular cases—there is need for development of novel materials for 3D printing, dedicated to the medical applications (e.g. in anti-allergic, water-resistant, non-fragile, etc.). Despite efforts of scientists traditional natural materials (wood, leather, and metals) may be hard to replace, and there will be necessity to incorporate these materials [1].

The paper presents the concept and implementation of innovative methods of producing artificial organs based on 3D printing technology. These organs possess physical and mechanical properties similar to human organs and bodies. As a result, it is possible to conduct training and workshops in the field of urological surgery, under the conditions close to real operations. Due to the fabrication of 3D models can also lead so-called pre-operations in order to better prepare surgeons to carry out complex operations and post-operation e.g. observers proper operation.

2 Genesis

Early studies by Wilson and Boland showed a cell printer placing cells in a shape similar to their respective positions in organs. This way printing of two-dimensional (2D) tissue constructs has been possible. Three-dimensional (3D) organ printing has been possible thanks to generation sequential layers for cell printing using thermo sensitive gels. Cells have been placed on previously printed successive layers. Researchers demonstrated optimal thickness of the gel, size of cells ensuring a direct contact between printed cell, and aggregation of the closely-placed cells in two types of thermo sensitive 3D gels [2, 3]. Several devices for:

- creating stable, functional protein arrays,
- positioning organic molecules, molecular aggregates, cells, and single-cell organisms onto solid supports,
- dispensing protein or cell solutions instead of the ink,
- creation of cell libraries as well as cellular assemblies that mimic their respective position in organs, have been developed using inexpensive high-throughput technology, fully automated and computer controlled [4]. Further development recognized four basic categories of jet based approaches to printing cells.
- laser guidance direct write (LG DW, since 1999)—to print formed embryonic-chick spinal-cord cells on a glass slide,
- modified laser-induced forward transfer techniques (LIFT),
- modified ink jet printers,
- electro-hydrodynamic jetting (EHDJ) method [5].

Direct neural tissue printing technique in a 3D multilayered collagen gel harness a layer of collagen precursor printed as a scaffold for the cells, and embryonic neurons and atrocities were subsequently printed on the layer. Sodium bicarbonate applied to the cell allowed the gelation of the collagen, and aforementioned process was repeated layer-by-layer [6]. Producing of the soft scaffold and placement of various cell types should be possible using a single 3D printing device. Boland et al. described spatial and functional controlled structure: simultaneous printing of cells and biomaterials with precise placement of cells and proteins within 3-D hydrogel structures. Various types of cells can be used, and capillaries and vessels can be also constructed within the scaffolds [7]. In the study by Kundu et al. multi-head deposition system was applied to fabricate 3D cell-printed scaffolds using poly-caprolactone and chondrocyte cell-encapsulated alginate hydrogel [8]. Ex vivo 3D engineering of functional tissues by laser-assisted bioprinting was also presented by Koch et al. (there were printed fibroblasts and keratinocytes embedded in collagen) [9]. The Zhang et al. printed human umbilical vein smooth muscle cells [10]. Inclusion of a vascular network to support cell viability required fabrication of printable vessel-like microfluidic channels supporting mechanical integrity ad enabling fluid transport [11]. In the study by Christensen et al., 3D printed vascular-like trees were fabricated thanks to liquid support-based inkjet printing approach (with calcium chloride as cross-linking agent and supporting material)

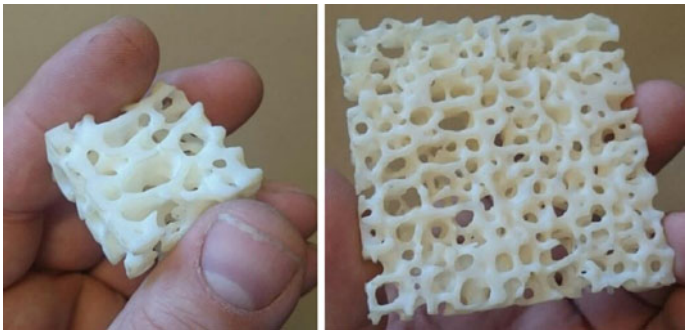

Fig. 1 Enlarged 3D reconstructions of trabecular bone, PolyJet 3D printing (made in Institute of Mechanics and Applied Computer Science)

[12]. Optimization of printing parameters allows for increased cell viability and mimics physiologically relevant attributes (e.g. cells density) of printed implant such as human skin [13]. 3D bioprinting technique may offer new possibilities for neural tissue regeneration. Next step is integration of 3D printed biological tissues with functional (micro- and nano-) electronics, such as bionic ear by Mannoor et al. [14]. Concept of 3D printed modular lung assist device was recently proposed by Rochow et al. [15]. Taking into consideration recent advances in brain-computer interfaces and neuroprosthesis, limitations of such approaches development are hard to overestimate. Study by Faulkner-Jones et al. showed printed human induced pluripotent stem cells [16]. Novel technologies represent 3D bioprinting of living soft tissue with cells on nanofibrillated cellulose. It is characterized by fast cross-linking ability of alginate and relatively high cells viability [17].

Current 3D bioprinting offers many possibilities to build functional biological tissues. In the future it may provide quick development toward on-demand individualized biological organs. Such situation will allow for optimization of complex therapy, even requiring modification or re-designing organic compounds for patients. Computer modeling and just-in-time production will extend aforementioned possibilities (Fig. 1).

3 Requirements

Regenerative medicine aims at:

- improvement,
- restoring,
- or replacement of damaged tissues/organs.

This aim is achieved using a combination of materials, cells, and growth factors. There are observed discrepancy between needs and possibilities. First of them is a gap between transplantation needs and tissues/organs shortages. High number of

elderly, severely ill and disabled people requires novel solutions, quicker and cost-effective. Artificial tissues and organs may deal with this problem. Future clinical use of 3D printed organs shaped the main requirement use on a large scale to manufacture tissues and whole organs. Detailed requirements are following:

- cell viability (requiring appropriate conditions cell dispensing and optimum concentration of alginate),
- retained genotype and phenotype,
- minimal stresses and forces encountered by cells during printing (cells should not be harmed by the printing procedure),
- precision, shape, structure, and function fitted to the original organ (e.g. investigation of cell localization and proliferation, and other characteristics),
- direct bioprinting of complex media exchange networks,
- (bio) mechanical properties and their change in time,
- as minimal as possible risk of graft rejection (including pre-programmed biocompatibility and biodegradability similar to the natural tissues), minimal adverse effects caused by biomaterial degradation,
- high precision, including minimal axially-varying deformation and uniform diameter along axial directions of 3D printed vessels,
- high automation and high flexibility of production,
- lack of post-fabrication procedure.
- Development of 3D organ printing generated three main directions of theoretical and practical (clinical) research:
- replicas (surrogates) of the tissues for learning and medical simulation purposes (e.g. preparation to the rare or complicated (neuro) surgical intervention),
- artificial implants of bones (including their complex internal porous structure),
- artificial implants of soft tissues—complex 3D organs (exact replicas of the natural organs of the particular patient or improved versions) with computer-controlled, exact placing of different cell types, completed even in several minutes, useful in applications relating to medical interventions and organ replacement [2, 3, 7].

Development of engineered tissues can be based on anatomic geometries derived from medical imaging modalities (microcomputer tomography and magnetic resonance imaging). The aforementioned approach provides precise artificial patient-tailored solutions (so called custom-printed), even characterized by very complex geometry [18]. They are regarded as easier to use than conventional brain atlases, often difficult to interpret, interface with printing system, and shape to the particulate patient needs [19]. Preoperative planning and in constructing personalized prostheses for patients are crucial for life-saving interventions [20]. Such phantom surrogates are often made from agarose gels of variable concentrations [21]. Complex structures based on a digital blueprint can be created using patient imaging data, so precision is guaranteed, even despite therapeutic modification and use of diverse materials. Both metal and synthetic implants, as far as 3D printed combinations of cells, growth factors and biomaterials (bioprinting) are currently

used in clinical practice, but number of limitation is huge and aforementioned technology still remains during its initial stage [22]. Chang et al. showed 3D-printed scaffold coated with mesenchymal stem cells (MSCs) seeded in fibrin for the repair of shape and function of reconstructed trachea [23]. Hsieh & Hsy showed 3D bioprinting neural stem cells embedded in the thermoresponsive biodegradable polyurethane [24]. 3D printers may be also useful for initiation of pre-programmed therapeutic chemical reactions purposes [25].

4 Technological Challenges

Bio-mimicked tissue/organs (including vasculature, muscle, cartilage, and bone) may be printed with capacity of single cell manipulation, digital control, high throughput even thank to the most popular bioprinting based on thermal inkjet. Biphasic or triphasic tissues are also available [26]. Computational modeling complexity may vary depending on many factors. Wang et al. studied such parameters of 3D printing as concentration of synthetic polymer solution (optimal: 12%), nozzle speed, and extrusion rate critical for implant quality [27]. Gao et al. studied the concentration and flow rate of the sodium alginate and calcium chloride during bioprinting. Higher concentrations of sodium alginate accompanied by smaller distance between adjacent hollow filaments allowed for printing high-strength structures with built-in microchannels [28].

Suy et al. described an agent-based modeling method, which uses the kinetic Monte Carlo algorithm as efficient time-dependent simulation tool to study the evolution of the multicellular aggregate system [29]. Cheung et al. developed a 3D printed realistic pediatric pyeloplasty simulator for educational tool (laparoscopic training and skills acquisition) [30].

The main technological problem is the process of tissue self-assembly and extracellular matrix deposition. Another technological barrier to overcome is building 3D vascularized organs. Skin, cartilage, or bladders are characterized by relatively simple structure, morphology, low oxygen consumption, and low requirements for blood vessels. More complex organs (heart, kidney, liver) are huge challenge for tissue engineering due to their complex cross-sectional vascular structure, diverse small diameter, unique mechanical properties and organization. Vascularization provides enough nutrients, waste removal, and gas exchange, required for maturation during perfusion. Thus many technologies were developed for vascularized tissue engineering, including cell sheet conduits, biodegradable synthetic polymer-based constructs and natural biomaterial-based blood vessel constructs (biomimetically fabricated bifurcated vessels, embedded micro-fluidic networks). Traditional scaffolds provide mechanical support of extracellular matrixes, but novel approaches provide both mechanical supports to cellular assembly and controlled fluid transport to and from the cells. Tissue spheroids or cell-encapsulated micro-beads may be used. Establishing connection of the implants to the host's vasculature maintaining implant' cells viability is also challenging task [31].

Precision is limited, but current achievements allow for 3D printing of millimeter to micrometer structures. With an development of the technology may be observed increase in the demand for increased precision of tissues and organs, especially printed form various materials (even mixed, e.g. biocells, plastic and metal) in room temperature. Moreover increases of aforementioned precision have to be accompanied by the novel miniaturized, biocompatible 3D systems (even joining printed and traditional technologies) [32].

Integrity is key value in the case of bio-implants. Very useful are compartmental studies analyzing analyze the successful interaction between the cells and the biomaterials (number of cells attached to scaffold) [33].

Two another major technical challenges have to be overcome:

- limited variety of 3D printable biomaterials not covering physical, chemical, and biological complexity and diversity of tissues and organs,
- combining of the scaffold structure with various in vitro and in vivo materials [34].

5 Own Research

3D printing can use various materials (plastic, metal, ceramics, living cells) in layers to produce a 3D object. There are many technologies, their features (speed, resolution) and materials, but general rule is the same: object is build vertically layer by layer allowing creation of the complex structures. In our investigation we have been using plastic as primary material, using FDM and PolyJet technology.

The proposed method enables the production of artificial human organs whose consistency, plastic properties, hardness, elasticity are close to the real organ of specific patient, because it can be made on the basis of the data from CT, MRI and 3D scans. The process of preparing 3D geometry is prepared in applications in the field of CAD, but also through advanced applications designed for editing in vector geometry environment (Figs. 2 and 3).

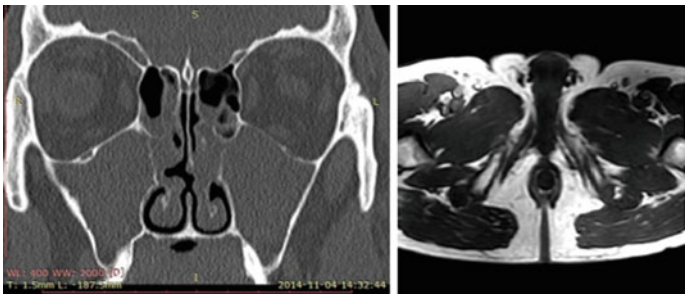

Fig. 2 CT 2D cross section (CS) image of human skull and human torso CS MRI image

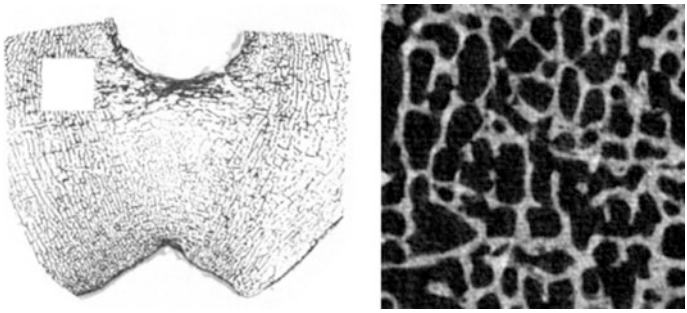

Fig. 3 Scan of micro Computer Tomography (μCT) and part of 2D layer of μCT data

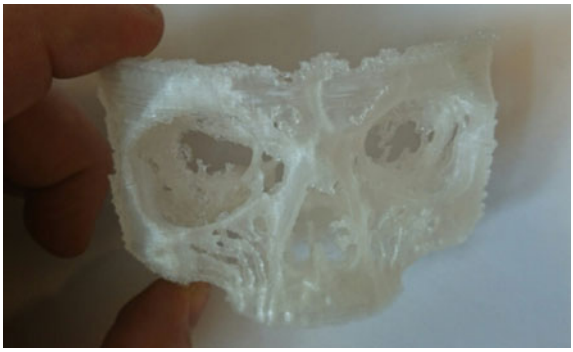

Fig. 4 3D printed skull reconstruction based on 2D cross sections CT technique (made in Institute of Mechanics and Applied Computer Science)

These organs possess physical and mechanical properties similar to human organs and bodies. As a result, it is possible to conduct training and workshops in the field of urological surgery, under the conditions close to real operations. Due to the fabrication of 3D models can also lead so-called pre-operations in order to better prepare surgeons to carry out complex operations and post-operation e.g. observers proper operation (Fig. 4).

We started to build digital library of particular human "spare parts", primarily bones, starting from bones of spine and breasts geometry form 3D scans. Such libraries (equivalent of the traditional anatomical atlases) are necessary for further developing the technology toward simplified and automated process accessible for non-professionals. Pre-programmed natural- or large-scale models of bones will be also useful for learning purposes, especially within medical simulation environments. Physiological and pathological models can reflect various age, deformations, and stage of the disease. Colored parts make easier recognition of particular bones. We hope open-source scientific libraries, inexpensive software, hardware and technology cause medical specialists familiar with this technology, and will have wide-reaching effects on the practice of medicine (Fig. 5).

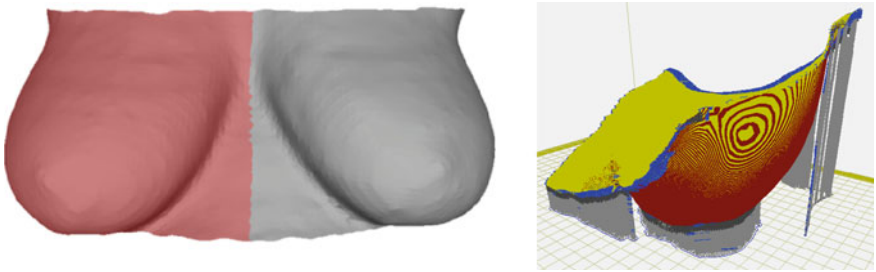

Fig. 5 Breast geometry from 3D scan and CAM visualization of mold geometry, with supports

6 Discussion

We are aware that replacement of the organs within human body has its own limitations. Its main aim is life saving. Even fully artificial bionic body (or body replacement) may not provide immortality due to nervous system degeneration.

Main limitation of the recent studies is usually initial stage of research and development. Proposed solutions are effective, but rather need for further research allowing for unification, automation, quality assessment, clinical trials (especially randomized controlled trials engaging huge groups of patients), compartmental studies with concurrent approaches, and finally clinical guidelines and commercialization. Their full clinical introduction may last at least several years (if will be cost-effective). We aim at early preparation of medical staff and current education system to novel solutions. Introduction of subsequent novel approaches may be this way regarded rather as procedure similar to the introduction of the novel surgical tool, but not novel approaches at all. Moreover application of 3D printed solutions may shape social awareness and make this solution familiar and expected both by clinicians and patients. Aforementioned situation may stimulate intensive research, quicker development, and earlier clinical application of the particular solutions. Maturation of the used technologies will foster increased publish confidence.

Limitations of the own concept is high initial cost of the whole system for clinical purposes, but low-cost 3D printing solutions may cause revolution concerning prices and provide better cost-effectiveness of the proposed solutions. Medical specialists seem be prepared for such novel technologies. Study by Jones et al. showed, that models fabricated using reverse engineering was useful for 95.8% (82.6% required models' price less than $500). Opinion that the models may be useful into the medical school curriculum was common [35].

There are still a few studies and publications. Recent review by Beuermeister et al. identified 1092 articles concerning clinical applications of 3D printing, but only 103 (9.43%) were included in the review. Despite strict selection topics of the selected papers were diverse as follows: general issues, surgical planning,

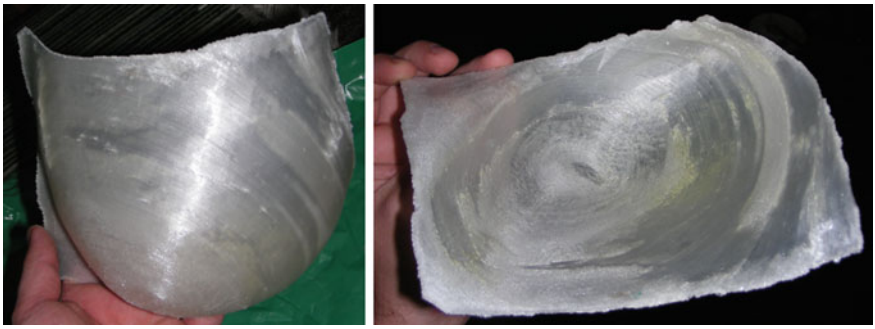

Fig. 6 Breast mold based on 3D scan, front and back view

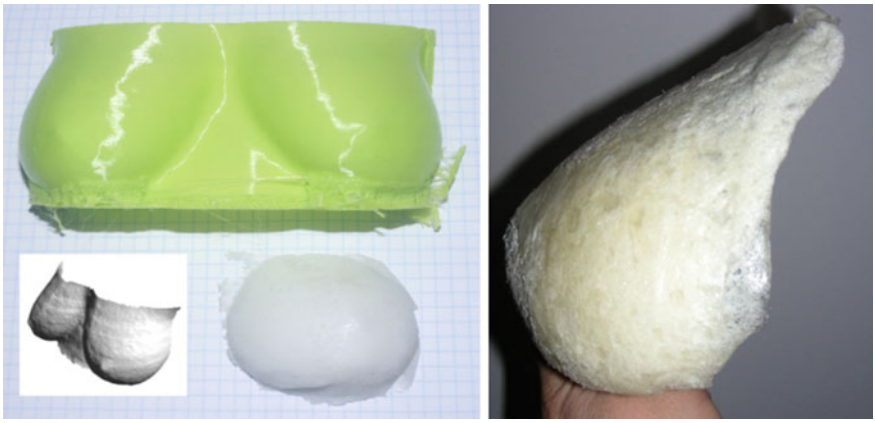

Fig. 7 Breasts mold after 3D print, 3D scan geometry, silicone breast replica in 1:3 scale and soft polyurethane breast prosthesis (made in Institute of mechanics and applied computer science)

training and patient education, upper extremity and hand prosthetics, bone reconstruction, breast reconstruction; nose, ear and cartilage reconstruction, skin [36] (Figs. 6 and 7).

7 Direction for Further Research

3D printing if tissues and organs has emerged as a new solution solving problem of increasing demands for organ transplants. Current findings are promising, 3D in vitro models and tissue substitutes may be useful for many clinical applications. But this way of tissue engineering still needs for further development. No doubts further challenges are:

- learning system of medical staff and translation technical solutions into clinical language,
- comparing and contrasting the different techniques and their properties,
- comparing and contrasting the different materials, including complex, heterogeneous tissues, monitoring of gap junctions, critical for tissue morphogenesis and cohesion,
- vascularization of the tissues, including planning and assessment of the fluid diffusion rate across the printed channels,
- innervations of implants,
- regeneration/replacement of complex tissues (heart, kidney, liver, etc.),
- survival rate of ex vivo cells in tissue grafts [37],
- accessibility and cost efficiency,
- indications and contraindications, adverse effects,
- specific therapy, rehabilitation and care guidelines concerning patients with various syndromes and injuries with diverse kinds of 3D printed implants,
- task division and co-operation within multidisciplinary therapeutic team.

The complicated nature of the 3D printing technologies and human organs require also:

(1) legal regulations,
(2) ethical regulations,
(3) patenting and licensing issues (e.g. who is the owner of rights to the particular additional copy of tissue/bone/neuronal structure: patient or anyone else) [38].

8 Conclusions

Artificial organs fabrication based on 3D printing technology is an innovation that is transforming the clinical practice. Its application expanded dramatically over the last 20 years. Many technologies were intensely investigated for possibilities to produce implants, tissue scaffolds, organs, and even surgical tools. Printing of heterogeneous 3D scaffolds built cell-by-cell may advance tissue engineering. Future, commercial techniques need to be better known, developed, and optimized toward printing the fully clinically functional tissues.

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