NEW TECHNOLOGIES AND BIOMATERIALS FOR THE GTR AND GINGIVAL PLASTIC SURGERY. REVIEW.

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Abstract

Periodontal treatment of tissue defects which comprises guided tissue regeneration and mucosal plastic surgery still remains nowadays a challenge in the dental office due to the lack of absolute predictability. New treatment techniques and biomaterials have emerged in order to counteract the different risks generated by the periodontal surgical methods. 3D and 4D printing technologies offer new possibilities in terms of patient-tailored materials, with lower complications and trauma and with the promise of a more accurate treatment plan and success.

Keywords: *periodontal guided tissue regeneration, barrier membrane, gingival graft, 3D printing, bioink*

Introduction

The deep and superficial periodontium comprises superficial and supporting tissues of the teeth which are composed of gingival tissue, cementum, periodontal ligament (PDL) and alveolar bone. Periodontitis is an inflammatory disease that leads to the degradation of periodontal tissue, with irreversible damage, causing tooth mobility and possibly tooth loss. Currently, the gold standard for periodontitis treatment focuses on plaque removal and local control of inflammation [1]. These therapies try to minimize symptoms and prevent the progression of the disease, but they cannot restore the attachment of periodontal tissues to the teeth and the restoration of periodontal tissues *ad integrum*. Therefore, the functions of the teeth and dentition remain affected after treatments. Some regenerative approaches, such as guided tissue regeneration (GTR) and bone grafts, have been developed to achieve periodontal tissue formation. However, the clinical outcomes of these approaches remain variable and unpredictable [2].

Oral soft tissues are complex biological systems that protect the oral cavity against exogenous irritants, substances, pathogens and mechanical stress. Tooth loss, disease, trauma or congenital disorders can lead to various oral soft tissue defects. When determining the type of treatment, special considerations must be observed so that chewing, speech and aesthetics can be properly restored and / or maintained. In restorative dentistry, remodelling and resorption of vestibular / oral bone after tooth extraction is a major problem [3].

Tissue engineering and regenerative medicine are interdisciplinary fields in order to develop procedures and technologies for the regeneration and / or

replacement of diseased or missing tissues. Today, advances in biomaterial science and production technologies allow the manufacture of intelligent, biocompatible polymers that can be combined with cells and signalling molecules to regenerate a multitude of tissues, including the oral mucosa and gingival tissue.

Three-dimensional (3D) printing is a new technology that has seen an astonishing expansion in recent years and opened new directions in regenerative medicine [4]. 3D printing methods allow the production of an individualized 3D object based on a defined shape, a biomaterial of your choice and a specific computer-aided design.

The ability to co-print living cells in a hydrogel has taken 3D printing to another level, opening up various possibilities for creating patient-tailored tissues. The main advantage of 3D printing approaches is the accuracy of the deposition of biomaterials and cells to produce a predefined shape, as well as to mimic the complex architecture of the tissue [5]. The very natural tissue organization achieved can improve the functional characteristics of the tissues after in vitro culture and / or in vivo application and can accelerate tissue regeneration.

Strategies in the regeneration of periodontal structures

There are two main strategies in periodontal regeneration: guided tissue regeneration (GTR) and tissue engineering techniques. GTR has been widely used to regenerate the periodontium for decades. It is a regenerative surgical technique that involves the procedure of a muco-gingival flap around the affected teeth, scaling and root planing and placement of barrier membranes under the flap, with or without various bone regeneration materials (Figure 1). The biological basis of the GTR technique is to block the apical growth of the epithelium to the space on the surface of the bare root by using the barrier membrane, thus facilitating periodontal ligament (PDL) cells and osteoblast to form PDL tissues and alveolar bone [6]. Numerous clinical trials have confirmed the benefits of GTR treatments, including a higher increase in clinical achievement (CAL), reduced probing pocket depth (PPD), and bone regeneration compared to single open flap debridement treatment (OFD) [7, 8].

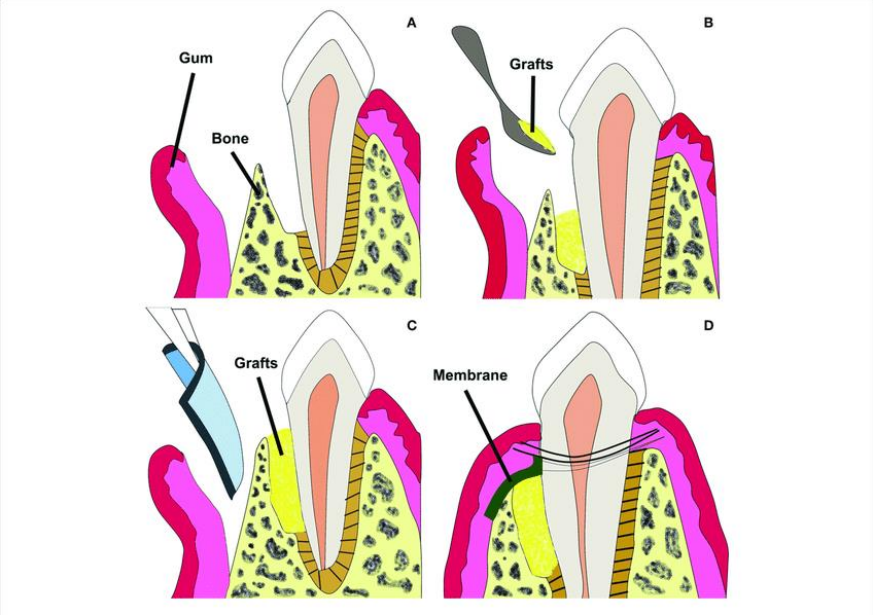

Figure 1. Basic principle of GTR [9, Yu Z-2019]

However, there are limitations in the treatment of GTR. First, the regenerative benefits of GTR treatment vary from case to case, depending on the existence of different risk factors and absolute / relative contraindications. Many factors, such as smoking, diabetes, improper control of dental plaque, variations in the anatomy and morphology of the teeth, can affect the predictability of GTR treatment [7].

Therefore, the results of GTR treatment in clinical practice may not be as successful as in clinical trials. A quantitative analysis showed that GTR was a predictable method for narrow infra-bony defects and class II mandibular furcation defects. However, the results of GTR treatment for other types of periodontal defects are limited and unpredictable [7].

GTR therapy involves the use of a membrane that acts as a barrier against epithelial cell migration, a process that can compromise the entire procedure. There are two types of barrier membranes for GTR: non-resorbable and resorbable membranes. For the membrane that cannot be resorbed, a second surgical step is required to remove it from the defect area, which increases the risk of infection as well as surgical trauma [10].

Tissue engineering techniques use stem / progenitor cells, scaffolds and bioactive molecules to build biomimetic systems to induce *de novo* tissue formation. Tissue engineering strategies for periodontal tissue regeneration can be classified into scaffold-free and scaffold-based techniques. For the scaffold-free technique, the cells or cell aggregates are transplanted to a defective area without a cell carrier. Several cell types, including bone marrow-derived mesenchymal stem cells (BMSC) [11], adipose-derived stem cells (ADSC) [12], periodontal ligament stem cells (PDLSC) [13], and pulpal stem cells (DPSCs) have been tested for the potential to form periodontal tissue. Direct cell implantation faces the problem of cell diffusion outside the defective area. The cell foil technique, which captures cells in the extracellular matrix (ECM) secreted by the cells themselves, is able to prevent cell migration. Cell foil therapy has been reported to induce more bone formation than cell suspension in porcine periodontal defects [14].

However, cell foil technology is able to regenerate only a layer of tissue with a simple structure. Given the complicated architecture of periodontal structures that includes two hard tissues (alveolar bone and cement) and a soft tissue (PDL), the use of the scaffold-based approach is the only choice for regenerating the PDL-cement-alveolar bone complex. Multiphase scaffolds with distinctive feature in each layer are needed to mimic periodontal structures.

Specifically, the architecture, chemical composition, cellular / biochemical composition of each layer must be adapted to achieve the regeneration of the periodontal complex [15].

Biomaterials used for barrier membranes

GTR barrier membranes prevent the growth of epithelial cells and provide space for PDL and alveolar bone regeneration. Barrier membranes should have basic properties, including biocompatibility, cell occlusion, tissue integration, spatial maintenance, and clinical maneuverability [16]. GTR barrier membranes are classified into non-absorbable and absorbable membranes. In general, non-absorbable membranes have a higher maintenance of space compared to resorbable membranes.

Gore-tex® is the first GTR barrier membrane made of polytetrafluoroethylene (PTFE) with high mechanical properties (Figure 2). The titanium-reinforced PTFE membrane has further increased the compressive strength, resulting in better results compared to the PTFE membrane [36]. The thin (0.01 mm) titanium-reinforced PTFE membrane took up

minimal space, thus providing more space for the formation of new tissues [17]. The smoother surface of titanium-reinforced PTFE also reduced the in vivo immune response. However, a second surgery is required to remove the non-absorbable barrier membranes, which increases the risk of infection, delays wound healing and affects regenerative outcomes [17].

Resorbable membranes are gradually degraded in vivo and avoid the disadvantage of secondary surgery after implantation. Both natural and synthetic biomaterials are tested as absorbable GTR membranes.

In general, natural biomaterials have excellent biocompatibility with cell binding sites, but lack mechanical strength [18]. On the other hand, synthetic biomaterials have variable degradation rates and mechanical properties, but no biological recognition (reason for cell binding). The degradation property of membranes affects the ability to maintain space and the formation of new tissues. As a rule, the rate of degradation should be moderate: rapid degradation leads to premature mechanical losses, while slow degradation prevents the growth of new tissues. In general, compared to non-absorbable membranes, resorbable membranes have a limited limitation of mechanical strength.

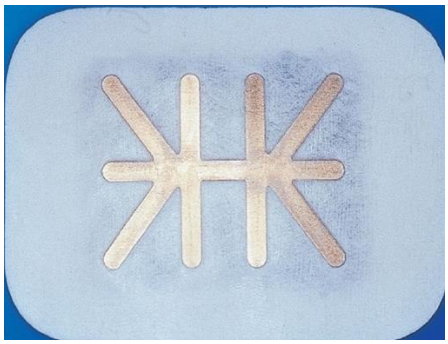

Figure 2. Gore-tex® membrane

The recent development of barrier membranes focuses on optimizing mechanical and degradation properties and incorporating new functions into GTR membranes. For example, GTR membranes have been prepared from composites that combine the advantages of different biomaterials [18]. The combination of natural and synthetic polymers has integrated the bioactive recognition of natural materials and improved mechanical properties of synthetic materials [19]. GTR membranes have also been used as carriers for drug administration to facilitate tissue regeneration [20]. Antibacterial drugs have been loaded into GTR membranes to inhibit local infection and inflammation, thus helping to form PDL tissue [21]. Multilayer GTR membranes with different functions in each layer have also been developed to improve the regeneration of periodontal tissue [22].

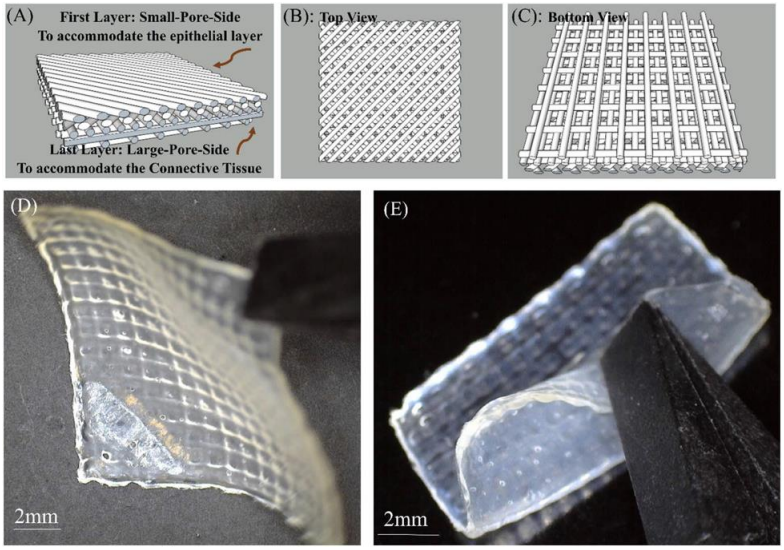

Fig. 3. (A–C): Schematic of 3D printed membrane design. (D-E) The macroscopic view of the 3D-printed gradient membranes of gelatine/elastin/sodium hyaluronate [23, Tayebia L-2018].

An interesting development includes the use of 3D technology in the creation of printed membranes. This is the product obtained by Tayebia et al. [23] The membrane is composed of 6 layers. The angles from the first layer to the last layer are: 45,135, 0, 90, 0, 90°. The distances between the wires (from the middle of a wire to the middle of the adjacent one) are set to 0.6 μm for layers 1-4 and 0.9 μm for layers 5-6. The membranes are completely flexible and surgical handling is easy. The thickness is about 150 μm (Figure 3).

Regarding the design of the membrane, in a new configuration, in order to be able to effectively grow one type of tissue (e.g. epithelial tissue) on one side of the membrane and another type of tissue (e.g. connective tissue / bone) on the other side, a gradient membrane with different structures on each side was made. This pattern could mimic a real tissue interface that has different tissues on each side. Part of the membrane had very large pores (400-500 μm), so we could sow cells that could easily grow on the membrane. On the other hand, the pore size was smaller (50-150 μm) [23].

Experiments conducted by Tayebia showed that this pore size made a thin layer of barrier that prevented keratinocytes from falling to the other side, which is a necessary feature for the membrane to facilitate guided tissue regeneration (GTR). It is important to remember that maintaining membrane permeability is essential because it allows the diffusion of nutrients and the growth and differentiation of healthy cells. The crosslinking helped maintain the structural integrity of the 3D printed membrane. Based on their visual observations, improper handling of the membranes was difficult and they collapsed easily. Thus, the degradation resistance of hydrogels could be significantly improved by crosslinking.

Oral soft tissue defects: current treatments and limitations

Oral soft tissues play a vital role in the structure and function of the oral cavity. Mucosal defects caused by tooth loss, gingival recessions, infections or trauma require tissue reconstruction [24]. Soft tissue augmentation is commonly used to regain reduced or lost tissue in edentulous patients, to cover an exposed root or implant, to increase the thickness of oral mucosal soft tissue, or for the height of coronary soft tissue [24]. The treatment must respect the functionality: mastication, speech and aesthetics.

Depending on the location and need, different techniques are used. Today, the gold standard for soft tissue enlargement is an autologous connective tissue graft, which can be either a free gingival graft (FGG) or a connective tissue graft (SCTG) [25]. However, the use of an autologous tissue graft has several disadvantages and limitations: 1) the amount of tissue that can be harvested is limited because the height, length and thickness of the donor's site depend on the anatomical position and vary depending on the patient; 2) the harvesting technique can be a surgical challenge; 3) a limited amount of tissue can be obtained per intervention and 4) patients complain of prolonged post-surgical pain and numbness [26].

To reduce all the risks and complications caused by graft harvesting, soft tissue replacements have been developed (Figure 4) [27]. An ideal non-autologous graft for soft tissue augmentation requires certain qualities, such as biocompatibility, volume and mechanical stability, tissue biodegradability and integration, safe handling and low cost, without compromising on efficiency [27].

Freeze-dried skin allografts were among the first products introduced in mucogingival surgery. They were initially used as a substitute for FGG in combination with an apically positioned flap to enlarge keratinized tissue. Subsequently, allogeneic dermal replacements were initially introduced to cover full-thickness burn wounds to increase keratinized tissue, to cover exposed roots, to increase vestibular height, and to treat localized alveolar defects [28]. Unfortunately, the results were associated with high rates of contraction of the grafted areas and delicate clinical handling.

Moreover, histological analysis indicated a significant structural difference compared to gingival tissue [29]. To reduce scar shrinkage and enhance the healing process, the xenogeneic (porcine) matrix that replaced autogenic tissue was developed and evaluated to increase the width of keratinized tissue and cover gingival recessions [30]. Clinical evaluation indicated a substantial improvement in the width of keratinized tissue with similar results compared to FGG [31]. Another cell matrix derived from porcine dermis was also used for extensive growth of keratinized tissue, resulting in tissue contraction [32].

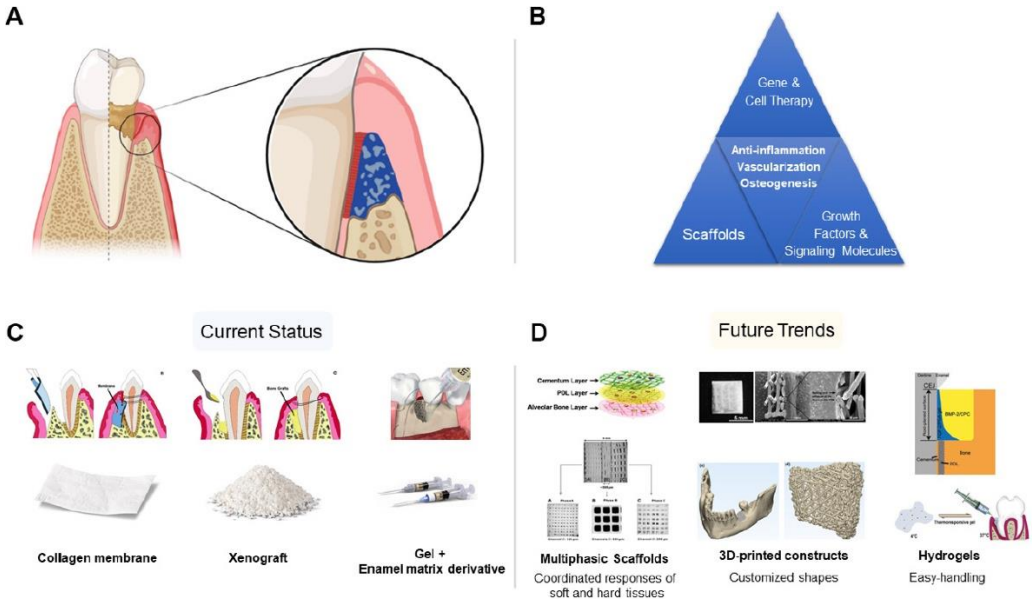

Figure 4. Present versus future in periodontal tissue reconstruction [33, Galli M - 2021]

An innovative, porous but volume-stable matrix of slightly crosslinked reconstituted collagen fibres was introduced, which increased the volume of soft tissues similarly to SCTG [34]. These biological scaffolds are promising for reducing morbidity, surgical time and costs for future oral soft tissue regeneration treatments. However, they are delivered in standardized shapes and sizes and must be adapted intraoperatively for each soft tissue defect, do not reproduce the precise architecture of the inner tissue of a particular oral site and remain surgically demanding.

A functionally and aesthetically pleasing solution for oral soft tissue augmentation thus remains a challenge. 3D printing technology could provide solutions for a better adapted, customized graft: 1) a 3D printed graft based on the shape of the individual defect with the tissue-specific inner structure produced on request in a centralized installation; 2) a 3D printed graft in the cabinet based on the shape of the individual defect with the tissue-specific internal structure 3) a 3D printed graft in the cabinet directly in the defect (in situ), based on the shape of the individual defect with the tissue-specific inner structure [35].

3D printing technology

3D printing allows the production of an individualized 3D object based on a chosen material and a specific computer-aided design (CAD). The process begins with the design of a 3D model, created by CAD software. The model is then converted into cross sections and sent to the 3D printer, which deposits layer after layer of the chosen material to produce the desired object. In the last decade, 3D printing technology has been widely used in various medical fields, including regenerative medicine, dentistry, the production of anatomical models and surgical guides, as well as in the pharmaceutical industry [36]. 3D printable scaffolds are used to build tissue models with or without cells, which allow the study of complex cellular interaction processes during tissue formation, maturation, and disease. Such tissue models are also used for drug screening and testing [37].

There are two main methods of printing established in the medical and dental fields: 1) indirect, where the scaffolds with the desired structure are printed to be later populated with cells and 2) direct, where the cells are co-printed within the scaffold ("bioprinting"). Hydrogels, in which cells live for printing, are called "bioink" [38]. Hydrogels are crosslinked hydrophilic polymers to form porous matrices with high water content, high biocompatibility and low toxicity [39]. To be suitable for 3D bioprinting, a hydrogel must be viscous enough for the printing process, but not mechanically damage the cells. On the other hand, you need to keep the 3D shape and structure when printing.

Hydrogels for soft tissues are used in four main 3D printing technologies: extrusion, laser-assisted, inkjet bioprinting and stereolithography [40]. In extrusion bioprinting, the pneumatic (pressure) or mechanical (piston) force extrudes the filaments, which must be subjected to rapid gelation to maintain the desired shape and structure. Laser-assisted bioprinting is based on a laser pulse that produces local heating of a solution containing cells, causing cells to fall in an orderly fashion on the other side of a platform / substrate. In inkjet bioprinting, a defined volume of fluid (with or without cells) is thrown onto a platform to obtain an accurate pattern. The droplets are deposited using either thermal or piezoelectric energy.

Stereolithography printing involves the light curing of light-sensitive polymers through precise light beam. The major advantage of extrusion and inkjet techniques is the construction of biomimic

tissue equivalents with complex cell loading using a multi-head approach with different cell types and biomaterials. The main disadvantage is that the bioactive cells or molecules must be in a liquid / semi-liquid state to allow deposition and subsequently solidify in the required structure [36]. The main advantages of stereolithography are high precision and constructive complexity, while the major disadvantage lies in the potential cellular damage generated by the light source [41]. Due to the exact positioning of different materials and / or cell types, the 3D printing approach will offer distinct advantages: the production of precise geometries to perfectly fit any defect and the faithful reproduction of the complex tissue architecture.

4D bioprinting appeared, where time was added to 3D bioprinting as the fourth dimension, to mimic tissue dynamics [42]. Based on intrinsic signals or in response to external stimuli, the 3D printed construction would change over time, resulting in a change in shape, structure and function. Similar to 3D printing, 4D printing also includes the approach of printing only biomaterial or biomaterial combined with cells [43]. 4D bioprinting is based on intelligent, receptive biomaterials, capable of changing when exposed to temperature, pH, humidity, electric or magnetic fields, light, sound or a combination thereof [43]. In addition, changes at the cell level, such as the response to coating / encapsulation, self-organization and / or deposition of the new matrix with simultaneous degradation of biomaterial can trigger changes that lead to more dynamic tissue [42]. An important aspect, especially for 4D printing, is the necessary mathematical modelling of the anticipated biomaterial transformation or cellular responses [44]. With the future development of smart biomaterials, 4D bioprinting will add to the possibilities of reconstruction of complex, heterogeneous tissues, including the oral mucosa.

Biomaterials used in 3D printing of soft tissues

Hydrogels offer a modifiable chemical composition and adjustable mechanical and biodegradable properties [45]. Hydrogel biomaterials can be classified into natural or synthetic derivatives. Natural biomaterials include agarose, alginate, collagen, gelatine, hyaluronic acid, chitosan, fibrin, cellulose and silk [46].

Synthetic biomaterials include polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), polycaprolactone (PCL), methacrylate gelatin, Pluronic®-127 or polyethylene glycol (PEG) and have been used in printing approaches. 3D of soft tissues [47]. The main advantages of synthetic polymers are the adaptation of the mechanical properties and degradation kinetics by changing the structure of the polymer. However, due to the lack of bioactive molecules, they are inefficient substrates for cell adhesion or migration [48]. To overcome the limitations of each group, different compound biopolymers have been developed and studied extensively [46, 49]. The bioprintability of hydrogels used for 3D printing of soft tissues is governed by their rheological properties and the targeted bioprinting technique (Figure 5) [47].

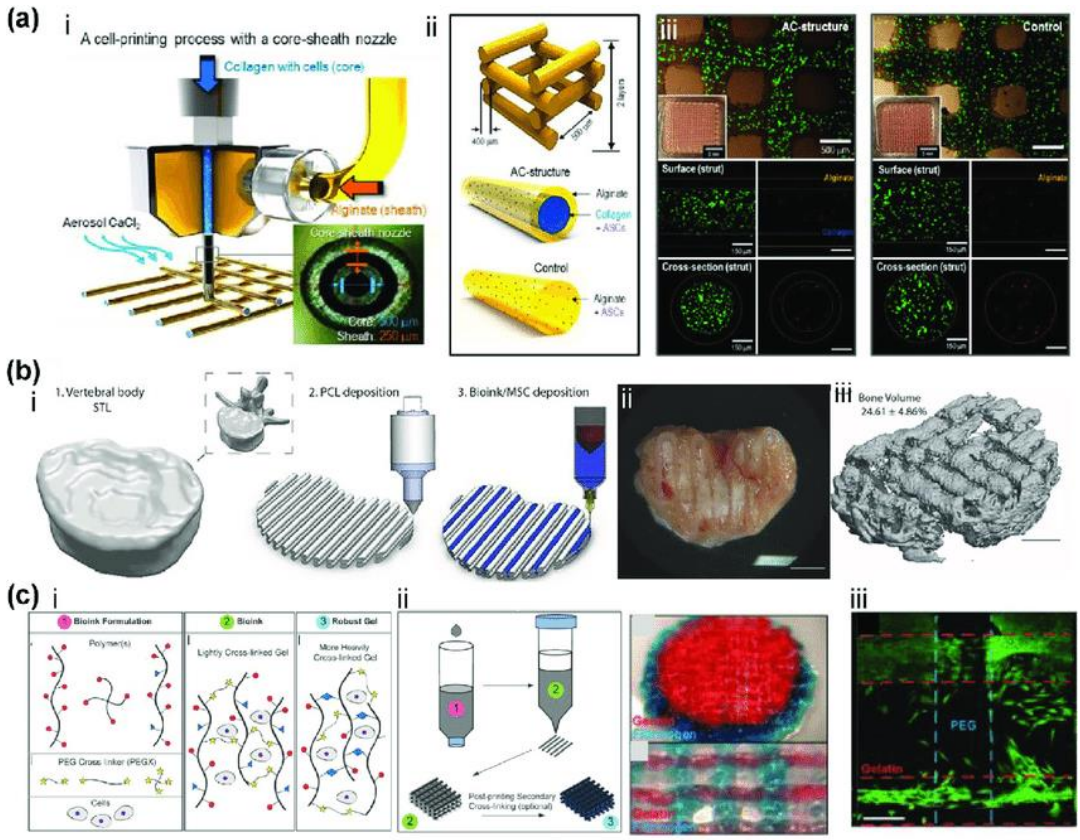

Figure 5. Spatially and temporally controlled bioninks [51, Han W - 2019]

In order to maintain the desired shape of the graft, the hydrogels must solidify. Cells can be seeded after gelation to avoid harsh printing / hardening conditions, or co-printed in hydrogel (bioprinting). Bioink gelling can be achieved by physical crosslinking (ionic, hydrophobic initiated with temperature changes or "self-assembly"), chemical crosslinking ensuring better mechanical stability (glutaraldehyde, genipine, irradiation-induced photopolymerization) or enzymatic (thrombin) crosslinking [47].

Various methods of solidification of materials, namely optical curing agents, UV, LED and lasers, are already present in dental clinics for the strengthening of restorative composites as well as binders. These methods would allow a smooth clinical translation for the soft tissue solidification of the stool, provided that they are compatible with the chosen hydrogels or cell-containing bioinks.

Bioinks for 3D printing can also be produced from decellularized matrix components [46]. Decellularized extracellular matrices have a major advantage: they contain all tissue components preserved in the correct proportions and tissue-specific signalling factors that together provide an instructive environment for cell migration, proliferation and differentiation [51]. An extensive analysis of bioink derived from decellularized pig skin, including concentration, viscosity, degree of crosslinking and cell viability, leads to the production of precise, 3D-printed skin-like constructs loaded with mouse fibroblasts [52]. Bioink derived from decellularized pig skin was bio-printed with human dermal fibroblasts to produce skin-like grafts [53]. The decellularized porcine small intestine submucosal suspension was developed and cryo-printed with rat dermal fibroblasts for skin regeneration [54]. Decellularization of gingival tissue has already been performed [55]. However, the production of a printable bioink will require modifications and adjustments.

Hydrogels allow the incorporation of instructive bioactive agents [56]. The presence of signalling molecules can guide the cells of the resident tissue, or it can provide the delivered cells with the necessary instructions for tissue regeneration. Hydrogel based on biphasic PCL / hyaluronic acid incorporating bone morphogenic protein 2 (BMP2) allowed in vivo bone formation [57].

A heparin-collagen gel containing BMP2 supported by 3D-printed bioceramic scaffolds induced osteogenesis of the MSC dental pulp in vitro and ectopic bone formation in a rat model [58]. A combination of BMP2 and vascular endothelial growth factor (VEGF) in a PLGA-PEG-PLGA hydrogel led to in vitro osteogenesis and bone regeneration in a rabbit model [55]. 3D printing of osteogen-peptide-loaded tricalcium phosphate / PGA coated with PLA and a collagen gel incorporating beta 1 growth factor (TGFβ-1) allowed the differentiation of MSC-specific cartilage and bone for bone marrow to regenerate osteochondral defects [59]. The presence of VEGF in a collagen gel induced endothelial cells to form capillary-like structures [60].

Alginate hydrogel incorporating PRP, known to contain a cocktail of growth factors, has been shown to improve endothelial proliferation and migration and MSC [61]. In another study, a scaffold composed of gelatin-sulfonated silk with built-in fibroblastic growth factor 2 (FGF-2) demonstrated improved host cell migration and superior wound healing and dermal vascularization upon insertion into a thick skin defect [62].

The development of such functionalized (instructional) biomaterials would facilitate regeneration based on patients' resident cells and could be particularly effective for oral soft tissues, given the high proliferation, migration and healing abilities of gingival fibroblasts [63].

Conclusions

While many GTR membranes have been developed for periodontal regeneration in recent years, most of them are in laboratory stages. Several in vivo and clinical studies are needed to evaluate the biosecurity and efficacy of these GTR membranes. The results of GTR treatment are unpredictable in several types of periodontal defects. In other words, GTR membranes with improved properties are insufficient to achieve successful treatment results. The combination of GTR membranes with other approaches, such as bone grafts, can provide better regenerative results.

Increasing gingiva / oral mucosa is a daily challenge in dental clinics. Although autogenous tissue grafts provide the desired results, their availability is limited. Moreover, the surgical procedure is painful and not without risks. Currently available graft substitutes do not provide satisfactory long-term functional and aesthetic results. The development of 3D bioprinting approaches would provide the manufacture of a graft shape perfectly adapted to the patient and a more natural inner structure of the oral gingiva / mucosa.

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