Methods to Enhance Mechanical Strength of 3D Printed Chitosan Scaffolds for Jawbone Regeneration

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Abstract

Current jaw reconstruction methods using autografts and allografts have limitations such as donor site morbidity, limited bone volume, and immunological rejection. As opposed to surgical methods synthetic biomaterials are used however they often fall short in providing the optimal combination of biocompatibility, mechanical strength, and customizable design required for effective jawbone reconstruction. 3D-printed chitosan scaffolds offer a promising alternative due to their biocompatibility, ease of customization, and potential for improved mechanical strength. This review explores strategies to optimize 3D-printed chitosan scaffolds for jaw reconstruction. We examine ways to increase their mechanical strength without compromising biocompatibility, with a particular emphasis on chemical modifications such as hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP) cross-linking and chitosan polyelectrolyte complex formation. We also discuss the critical roles that 3D printing processes (FDM and SLA) and intrinsic chitosan qualities (degree of deacetylation) play in making an ink printable. Optimizing 3D printing methods for chitosan-ceramic composites and exploring biocompatible additives to enhance printability are identified as key areas for further research. By addressing these challenges, 3D-printed chitosan scaffolds have the potential to become next-generation biomaterials for jaw reconstruction, revolutionizing the field through precise anatomical adaptation, enhanced osteoconductivity, controlled biodegradation, and the capacity for incorporating bioactive molecules, ultimately leading to accelerated bone regeneration and improved functional and aesthetic outcomes.

Keywords

Biomedical engineering; Cell and tissue engineering; 3D printing; jawbone regeneration; chitosan

Introduction

Facial trauma, severe cleft lip, and oral cancer collectively affect 2.2 million people annually and they have devastating impacts on thousands of patient's quality of life.[1–3] The current gold standard for resolving critical cases of these diseases include jaw reconstruction surgery, allogenic bone grafts, autografts, and vascularized bone flaps, and distraction osteogenesis.[4] However, these methods come with drawbacks such as risk of disease transmission and immune rejection, risk of inflicting secondary trauma, pain, elongated recovery, and limited restoration of full functionality.[5] Recognising this there has been significant attention surrounding synthetic biomaterials, however, they often struggle to achieve the ideal balance of biocompatibility, osteoconductivity, and mechanical strength needed for jaw reconstruction. This has fueled the search for alternative solutions that can offer faster recovery times, improved patient outcomes, cost-effectiveness, and greater design flexibility. One such solution is 3D printed chitosan scaffolds.

Chitosan, a deacetylated form of chitin, is a naturally occurring polysaccharide composed of β-(1-4)-linked N-acetylglucosamine (GlcNAc) and glucosamine (GlcN) units as seen in Figure 1.[6] The presence of a free amine group (NH_2) on the GlcN unit imparts a cationic charge to chitosan. This positive charge helps osteoblasts adhere

to the scaffold surface. This initial attachment is a critical step for cell proliferation and differentiation, ultimately leading to new bone formation.[7]

Chitosan exhibits excellent biocompatibility, making it well-tolerated by the body. The cationic charge of chitosan allows it to interact with negatively charged glycosaminoglycans (GAGs) present in the extracellular matrix of bone. This interaction facilitates cell adhesion, proliferation, and differentiation of the bone-forming cells called osteoblasts.[8] Additionally, chitosan is biodegradable by enzymes like lysozymes, allowing for the controlled release of growth factors or therapeutic agents embedded within the scaffold. Importantly, as the scaffold degrades, the body's own bone-forming cells can remodel the space and replace it with new bone tissue.[9] This controlled degradation allows for the gradual release of growth factors or other therapeutic agents potentially encapsulated within the scaffold, while simultaneously creating space for new bone tissue formation. Chitosan also possesses film-forming abilities, making it suitable for 3D printing into complex scaffolds with desired porosity and architecture.[10]

Figure 1. Illustrates chitosan's chemical structure and properties.

The jaw is a remarkable structure with a complex combination of properties that necessitates careful consideration when designing scaffolds for reconstruction. Since it experiences significant forces during everyday activities like chewing and speaking it is required to be mechanically strong to withstand stress. Furthermore, the jaw is constantly moving, this necessitates a material that is not only strong but also exhibits a degree of elasticity to accommodate this movement.[11] The compressive strength for jaw scaffolds is typically within the range of 4-20 MPa, depending on the specific location within the jaw (e.g., higher strength needed in the condyle compared to the alveolar ridge). It would also require an ideal pore size for bone ingrowth and vascularization is typically between 100-500 micrometers.[12] Finally, in order for the scaffolds to result in optimal patient outcomes it should exhibit minimal cytotoxicity and good cell attachment and proliferation, as assessed by *in vitro* cell viability assays to ensure biocompatibility and safety.

3D printing allows for creating patient-specific scaffolds with precise geometries that mimic the complex shape and structure of the jawbone as well as specific pore size and distribution control in order to promote cell-growth and blood vessel infiltration. In order for a material to be printable it must have a suitable viscosity, gelation property, and thermal properties as for certain printing techniques the melting point and thermal stability are crucial.[13] Chitosan's protonated amino groups allow its viscosity to be modified as the positive charge leads to electrostatic repulsion between chitosan molecules, causing them to expand and increase the viscosity. It also has a gelation property which allows it to become a gel under specific conditions. These factors make it printable, allowing for enhanced customization.

However, the primary challenge in making it more widely adopted is insufficient mechanical strength. While current reviews have explored the applications of chitosan for jaw reconstruction, drug delivery, and wound healing there has been limited exploration into chemical and 3D modeling methods to enhance mechanical strength while maintaining biocompatibility. This review overviews the current applications of 3D printed chitosan scaffolds and critically analyzes existing literature to optimize 3D-printed chitosan scaffolds for improved mechanical performance

and degradation control. By addressing these challenges, we can pave the way for the development of next-generation biomaterials tailored for jaw reconstruction, ultimately leading to improved patient outcomes.

Discussion

Chemical Methods for Mechanical Strength Enhancement

The jawbone is a strong and complex structure that needs to withstand various forces during chewing and speaking. A chitosan scaffold for jaw reconstruction ideally needs similar mechanical strength to provide proper function and support. In its natural state, chitosan is relatively weak and brittle, making it less suitable for load-bearing applications like jawbone reconstruction.[14] Hence, chemical methods such as cross-linking with Hydroxyapatite, beta-tricalcium phosphate as well as neutralization and forming polyelectrolyte complexes can enhance the mechanical strength of the material.

Hydroxyapatite (HA) is a biocompatible ceramic similar to the mineral component of bone and is a popular choice for composite scaffolds. The strong interface creates a more cohesive and robust structure within the scaffold. Furthermore, HA itself is a much stiffer material compared to chitosan. By incorporating HA particles, the overall stiffness of the composite scaffold increases. Studies have utilized methods such as X-ray diffraction and Scanning Electron Microscopy to analyze the morphology, crystallinity, and chemical interactions at the interface. Results from all methods generally showed that synthesized HAp exhibited preferential crystalline orientation in the [001] direction; this refers to a specific crystal plane in the HAp lattice. When the synthesis process favors this particular orientation, the atoms are arranged more uniformly, leading to "high crystallinity" - a more perfect and ordered crystal structure. Natural bone is a composite of collagen and hydroxyapatite crystals. By incorporating highly crystalline HA, the chitosan scaffold mimics the natural stiffness and mineral composition of bone.

Figure 2. Microscopic images of Chitosan-Hydroxyapatite Scaffold.

This enhanced biomimicry promotes osseointegration, the process by which new bone tissue grows into and bonds with the scaffold. A stronger and more bone-like scaffold provides a better template for bone regeneration.[15,15–20] As shown in Figure 2, an SEM image of chitosan-hydroxyapatite scaffold in-vitro there was significant stem cell proliferation, attachment, and differentiated lineages.

β-TCP, another bioactive ceramic, offers additional benefits. First, β-TCP particles act as a reinforcing agent within the chitosan matrix. These particles distribute stress throughout the composite, preventing cracks from propagating and leading to overall stronger material. β-TCP particles are dispersed throughout the chitosan matrix. When the composite experiences stress or force, these particles help distribute that stress more evenly throughout the material. This prevents the formation and propagation of cracks within the chitosan, a major factor contributing to scaffold failure. By effectively distributing stress, β-TCP reinforcement enhances the overall mechanical strength of the composite scaffold. This allows the scaffold to withstand the forces exerted during jaw function, making it a more suitable material for load-bearing applications in jaw reconstruction. In addition, the presence of β-TCP particles creates a more tortuous path for crack propagation within the scaffold. As a crack encounters a particle, it

needs to deviate around it, effectively increasing the energy required for the crack to grow. This increased resistance to crack propagation translates to a stronger composite material.[19,21]

Furthermore, chitosan can interact with the surface of β-TCP particles through hydrogen bonding or other mechanisms. This creates a strong interface between the two materials, allowing them to work together more effectively. A study by Bandyopadhyay et al. in 2018 explored 3D-printed chitosan/β-TCP scaffolds and reported enhanced compressive strength (up to 40 MPa) and improved osteoconductivity compared to pure chitosan scaffolds.[22] The authors suggest that β-TCP promotes apatite formation, mimicking the mineral phase of bone and facilitating stronger bonding between the scaffold and newly formed bone tissue. Additional studies conducted by Caicedo et al. show that based on XRD results, the level of coupling between β-TCP and chitosan can be observed, indicating the possibility of crystalline functionalization without crystallinity or structure loss when the chitosan content increases from 0% to 50%.[23]

Demirtas et al. presented a bio printable form of chitosan using B-GP for the first time. They successfully printed MC33-El preosteoblast cell-laden hydrogels by thermal crosslinking. Furthermore, they asserted that chitosan outperformed alginate in terms of cell function. The benefits of B-GP chitosan combination were also explored by Roehm et al, who optimized a 3D bioprinting approach using a chitosan-gelabelieve blend ink that included IMR-32 cells from neuroblastoma. Addition of B-GP to the bioink allowed hydrogel formation and body temperature without the necessity of post-printing processes. Furthermore, 3D bioprinted scaffolds demonstrated excellent cell viability and a homogenous cell distribution. This aligns with the previously mentioned ideals as not only was there positive biocompatibility from *in vitro* studies, but also an increase in crystalline function without structure loss.[24]

Another method is neutralization steps, which are usually accompanied by shrinkage processes which significantly reduce the fidelity and resolution of the printed scaffolds, to avoid this silk particles have been incorporated to improve mechanical properties and reduce post-shrinkage due to coagulation. Wu analyzed the effect of neutralization steps on the mechanical properties and printing fidelity of very intricate structures. They used a very concentrated chitosan solution (10 wt-%) that was dissolved in an acidic mixture, and 3D printed scaffolds were immersed in a 1 M NaOH solution as a coagulation bath. In this work, the authors were able to fabricate complex scaffolds with very high resolution (~ 30 µm).[25]

The formation of chitosan polyelectrolyte complexes is another interesting approach to improve mechanical properties of hydra protect based inks. Blending different polymers (e.g. gelatin, alginate) can provide additional features to the systems when they are used for individual formation of polyelectrolyte complexes and electrostatic interactions between the mixed polymers, which exhibit opposite charges. Liu et al. obtained 3D printed scaffolds from an alginate-chitosan polyion complex ink. The addition of chitosan to the polyanionic alginate increased the viscosity of the alginate solution. 3D structures with excellent shape fidelity and improved mechanical strength were obtained.[26]

Overview of practicality and printing process

The first crucial step in 3D printing chitosan scaffolds involves preparing the material. Chitosan is dissolved in an acidic solution, typically acetic acid, to create a printable ink. This ink needs a specific balance of properties. It must have a low enough viscosity to flow smoothly through the printing nozzle during the chosen technique. Additionally, the ink should exhibit shear-thinning behavior. This means its viscosity decreases as pressure is applied, allowing for easier flow during extrusion and regaining its shape after deposition for precise scaffold formation. Finally, and most importantly, for successful implantation in the jaw, the ink must maintain biocompatibility. Any additives used to improve printability cannot compromise the material's ability to be safely accepted by the body.[30]

Figure 3. Diagram of 3D printing preparation process for extrusion-based techniques.

Then, the material is either printed through extrusion-based techniques such as Fused Deposition Modelling as shown in Figure 3, Stereolithography (SLA) which uses a laser beam to selectively cure liquid chitosan, or other modeling techniques including electrospinning. There are a variety of factors that can be controlled during the 3D printing process which can affect printability and mechanical strength, as summarized in (Table 1).

Factor	Control method	Effect on Mechanical Strength	Mechanism[1]
Infill Density	Controlled through slicer software settings. Users can define a percentage value (e.g., 20%, 50%, 80%)	Higher density generally leads to increased strength	More material provides greater resistance to load
Infill Pattern	Selected from various options within slicer software. Common choices include grid, honeycomb, and rectilinear.	Grid or honeycomb patterns offer better load distribution	Interconnected structure enhances stiffness
Layer Height	Adjusted in slicer software settings. Lower layer heights result in thinner layers	Thinner layers improve interlayer bonding	Reduces weak points, increases overall strength
Build Orientation	Determined by the user during model positioning in slicer software. Parts can be rotated and aligned to optimize strength based on expected forces.	Aligning strongest axis with load direction	Maximizes material resistance to forces
Nozzle Temperature	Controlled through settings on the 3D printer itself.	Optimal temperature ensures good material flow and layer adhesion	Prevents defects that reduce strength
Post-Processing Techniques	Implemented after the printing process is complete	Improves crystallinity, reduces porosity, enhances surface finish	Increases material density and resistance to stress

Table 1. 3D printing settings, their effect on mechanical strength, and how to control them.

Beyond the initial solution preparation and chosen 3D printing technique, several factors inherent to the chitosan

material itself significantly influence its printability. These factors play a crucial role in determining how smoothly the chitosan flows during the printing process and how well it forms the desired scaffold structure. One key factor is the molecular structure of the chitosan. The percentage of acetyl groups eliminated from the chitosan molecule is known as the degree of deacetylation, or DD. Interestingly, higher DD chitosan, which has a greater number of positively charged groups, generally exhibits lower viscosity and improved shear-thinning behavior. This translates to better printability. The positive charges on the chitosan molecules repel each other, creating a natural tendency to resist close packing and flow more easily. Additionally, under pressure during extrusion, these charges can temporarily rearrange, allowing the chitosan solution to flow more readily and then return to its original structure once the pressure is released. This shear-thinning behavior is essential for maintaining a smooth flow through the printing nozzle while ensuring the material retains its shape after deposition for precise scaffold formation.

Gelatin-chitosan polyelectrolyte hydrogels were also utilized by Ng et al. to 3D print skin structures. Because of the two polymers combined, blend ink also showed enhanced viscosity, which made it easier to deposit complicated structures in three dimensions. UV-crosslinkable chitosan derivatives have been produced and used in 3D printing with success in recent years.[27] Sayyar et al. synthesized 3D printed conductive scaffolds by combining chemically transformed graphene with photocrosslinkable chitosan.[28] These scaffolds were also immersed in an isopropanol bath to achieve complete crosslinking. Saraiva et al. also used methacrylated-chitosan in combination with GelMA to obtain more stable structures and enhance mechanical properties derived from photopolymerization processes of both polymers. For example, printed scaffolds were biocompatible and supported surface cells adhesion and internalization.[29]

Methods to generate mechanically strong 3D chitosan structures for scaffolds

Fused Deposition Modeling (FDM), also known as Fused Filament Fabrication (FFF), is a popular additive manufacturing (AM) technique for creating three-dimensional (3D) scaffolds for bone tissue engineering. It offers several advantages in terms of mechanical strength, design flexibility, and biocompatibility, making it a promising technology for bone regeneration applications. The first step involves creating a computer-aided design (CAD) model of the desired scaffold. This model defines the scaffold's geometry, porosity, and internal architecture, which are crucial for mimicking the natural bone structure and optimizing mechanical properties. This allows for the creation of scaffolds with controlled pore size and distribution. This porosity plays a significant role in mechanical strength. By adjusting printing parameters, engineers can design scaffolds with varying degrees of porosity, balancing strength with factors like cell infiltration and nutrient exchange. Furthermore, FDM allows for the incorporation of different infill patterns within the scaffold structure. These patterns, such as honeycomb or grid structures, significantly influence the scaffold's mechanical behavior. Choosing appropriate infill patterns can optimize the scaffold's stiffness, strength, and load-bearing capacity to match the specific requirements of different bone types. [31,33]

Another commonly used technique is electrospinning. Electrospinning is a process that uses high Another commonly used technique is electrospinning. Electrospinning is a process that uses high voltage to create nanofibers from a polymer solution. A charged jet is ejected from a Taylor cone formed at the needle tip due to the balance between electrical forces and surface tension as demonstrated in Figure 4. The jet accelerates towards a grounded collector and forms fibers as the solvent evaporates. Different electrospinning setups and strategies, such as AC voltage and specialized collectors, can be used to produce various nanofiber structures. It is suitable for creating nanofibrous scaffolds with high porosity, ideal for all cell attachment and nutrient transport. Furthermore,

the fibers possess an exceptionally high surface area to volume ratio compared to conventional fibers or particles, allowing for increased interfiber interactions and improved load transfer, leading to enhanced mechanical strength. The electrospinning process itself also inherently creates highly porous structures with interconnected pores. While porosity is generally associated with reduced mechanical strength, careful control of processing parameters can optimize pore size and distribution to achieve a balance between porosity and mechanical integrity.

Electrospinning is suitable for creating nanofibrous scaffolds with high porosity, ideal for cell attachment and nutrient transport. However, achieving sufficient mechanical strength for jaw applications can be challenging due to the inherently weak nature of nanofibers. Electrospinning chitosan with other polymers (e.g., PCL) or biocompatible ceramics (e.g., HA nanorods) can enhance strength. However, controlling fiber diameter and achieving good structural integrity for jaw reconstruction remains a challenge. Optimizing electrospinning parameters and using collector designs that promote fiber alignment can improve scaffold strength.[34–36]

Figure 4. Electrospinning process used for chitosan.

A stereolithography setup consists of a container for photocurable liquid resin, a laser source (typically UV light) that causes the liquid resin to polymerize and cross-link, a system that allows the laser beam to move in the horizontal plane (X and Y directions), and a system that regulates the movement of the fabrication platform in the vertical plane (Z direction).When photocurable liquid resin is exposed to light in a two-dimensional pattern, it absorbs a single photon and solidifies to a predetermined depth, which is usually higher than the manufacturing platform's step height. This means that in the next layer, unreacted functional groups from the first photopolymerization layer will polymerize with the irradiation liquid resin. After curing, the fabrication platform travels in a Z-direction, layer by layer. This allows it to have a high resolution and precision, enabling the creation of complex structures with intricate features that mimic the natural jawbone. However, due to the incomplete conversion of reactive groups, post treatments of washing-off residual resin and curing with UV light are usually needed to promote mechanical properties. Nevertheless, further research is needed to optimize resin composition and printing parameters for consistent and reliable scaffold fabrication.[37–39]

Figure 5. Stereolithography printing set up.

Extrusion-based 3D printing is the computer-controlled layer-by-layer deposition of molten/semi molten polymers, polymer solutions, pastes, or dispersions through a movable nozzle acting as the extrusion print head in a direct ink writing mode. Figure 4 reveals the different mechanisms of ejection of ink materials. Extrusion printing can be divided into two classes of melting-based process e.g. fused deposition modeling and melt electrospinning writing and dissolution-based process e.g. 3D plotting. Based on 3D dispensing that was first developed as an AM method in 2000, 3D plotting provides a versatile extrusion printing technique. In this 3D plotting process, the extrusion print head consists of a nozzle and a cartridge, which can horizontally (X- and Y- directions) and vertically (Z direction) move through a computer-controlled manner. 3D dispensing is pneumatically controlled by altering air pressure. Extrusion-based 3D printing offers a versatile approach to enhancing the mechanical properties of chitosan scaffolds for bone tissue engineering. This technique provides precise control over material deposition, enabling the creation of scaffolds with tailored porosity, intricate geometries, and optimized chitosan concentrations. By adjusting extrusion parameters such as pressure, speed, and nozzle diameter, researchers can fine-tune the scaffold's microstructure and mechanical behavior.

Conclusion

This review determined that 3D-printed chitosan scaffolds offer significant promise for jaw reconstruction due to their biocompatibility, ease of customization, and potential for improved mechanical strength. Successful methods to improve mechanical strength were forms of chemical modifications incorporating biocompatible ceramics like hydroxyapatite (HA) and beta-tricalcium phosphate (β-TCP). These reinforcements provided stiffness and promoted aparitie formation, mimicking the natural bone composition and enhancing osseointegration. This review also emphasizes that the selection of 3D printing techniques also plays a crucial role in achieving optimal printability and final scaffold properties. Fused Deposition Modeling (FDM) and Stereolithography (SLA) were discussed, highlighting their distinct advantages and considerations for chitosan-based bioinks. Furthermore, the intrinsic properties of chitosan, particularly the degree of deacetylation (DD), significantly influence printability. Higher DD chitosan exhibits improved printability due to its enhanced shear-thinning behavior.

However, while 3D printed chitosan scaffolds offer promising possibilities for tissue engineering applications, future research efforts should focus on *in vitro* studies and novel methods to improve mechanical strength. One such avenue is microfluidics and Digital Light Processing (DLP). Studies investigating the feasibility of these techniques for creating chitosan scaffolds with well-defined microstructures that enhance strength and mimic the natural bone architecture are warranted. Integrating bioprinting with chitosan-based 3D printing could enable the co-deposition of chitosan and reinforcing agents within the scaffold, leading to superior strength and bioactivity. We recommend exploring bioprinting techniques that offer high resolution and biocompatibility for seamless integration with chitosan bioinks.[40–42]

Moreover, computational modeling tools like Finite Element Analysis (FEA) can be employed to optimize scaffold design by predicting their behavior under various loading conditions. Additionally, multiscale modeling can provide valuable insights into the interplay between scaffold architecture and microscale interactions. Future research should focus on developing robust computational models that can accurately predict the mechanical performance of chitosan scaffolds with various designs and compositions.[44]

Finally, *in situ* mineralization techniques hold potential for improving both bioactivity and mechanical strength. Introducing mineral precursors like calcium and phosphate ions into the bioink or employing post-processing methods can promote the formation of hydroxyapatite within the scaffold, mimicking the composition of natural bone. We recommend studies optimizing the concentration of mineral precursors and post-processing conditions to achieve controlled mineralization within the chitosan scaffold while maintaining its structural integrity.[45,46]

By exploring these future directions and recommendations, researchers can develop next-generation 3D printed chitosan scaffolds with enhanced mechanical properties, significantly expanding their potential for successful applications in bone tissue engineering and other demanding biomedical fields.

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