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A review: chitosan as 3D matrix for tissue engineering

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Abstract
Highly porous 3Dimensional (3D) scaffold becomes a promising alternative approach for tissue repairing. Tissue engineering (TE) is an interdisciplinary field involving principles of engineering and biological sciences to regenerate new tissue and organs using cells and scaffolds. Functional biomaterial research has focused on the development and improvement of scaffolding which has played an important role in the therapeutic approach in the current and future medicine. Scaffolds are one of the decisive factors for tissue engineering. It consists of natural polymers which have recently been developed more quickly and have gained more popularity. These include chitosan, a copolymer derived from the alkaline deacetylation of chitin. Expectations for use of these scaffolds are increasing as the knowledge regarding their chemical and biological properties expands, and new biomedical applications are investigated. Due to their different biological properties such as being biocompatible, bio adhesive, biodegradable, and bioactive, they have given the pattern for use in tissue engineering for repair and/or regeneration of different tissues including skin, bone, cartilage, nerves, liver and muscle. Here we highlight recent advances in the development of chitosan-based scaffolds with enhanced tissue regeneration capability.

Keywords: Chitosan, Scaffolds, Tissue Engineering

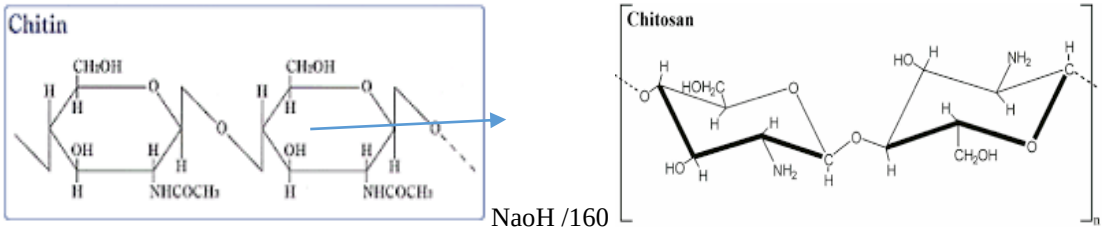
Introduction [1, 2, 4, 7].

Tissue engineering is an important field of research for developing new three dimensional (3D) scaffolds with highly interconnected porous structure. These scaffolds should match the characteristics of the tissue that is to be replaced. The tissue engineering scaffolds need to be biocompatible, bio adhesive, tissue inductive, tissue conductive, and mechanically well-suited in order to restore tissue which have been lost or damaged. In this respect, artificial scaffolds based on chitosan, inorganic materials, and/or synthetics polymers have received much attention in recent years. In tissue engineering, three-dimensional (3-D) porous scaffolds are used for cell adhesion, proliferation, and differentiation and development of an extracellular matrix (ECM). The tissue scaffolds may have an ability to load bioactive/therapeutic substances and to release them at a controlled manner in the defected sites. These extraordinary characteristics can be used to improve the bioactivity of the scaffolds and therapeutic effects in the injured tissues. For the betterment of tissue engineering approaches, more appropriate materials with the suitable cells and bioactive molecules need to be considered for the fabrication of scaffold. During the tissue regeneration, all of these factors like biocompatibility, bio adhesive, bioresorptivity, bioactivity, biodegradability, cytocompatibility, tissue inductive and conductive, mechanical stiffness, surface contact, haemostatic and inbuild antimicrobial properties play an very important roles in the healing process. In the field of bone tissue engineering three dimensional scaffolds facilitate the cells, augment and then regenerate three dimensional tissues. This unique property of Chitosan plays a significant role in fabricating highly porous scaffold which is intended to provide sufficient mechanical strength and will maintain structural integrity during *in vitro* / *in vivo* tissue remodelling process.

Chitosan structure and properties [3, 4, 5].

The chemical structure of chitin and chitosan is very similar to that of cellulose which consists of several hundreds to more than thousand b-(1-4) linked D-glucose units. In chitin and chitosan structure hydroxyl at position C_2 of cellulose has replaced by an acetamide group. Chitosan, b-(1-4) linked 2-amino-2-deoxy-b-D-glucopyranose, is an N-deacetylated derivative of chitin obtained by transforming the acetamide groups into primary amino groups. However, deacetylation of chitin is almost never complete and chitosan or deacetylated chitin still contains acetamide groups to some extent. Unlike cellulose, chitin and chitosan contain 5–8 % nitrogen, which in chitin is in form of acetylated amine groups and in chitosan in form of primary aliphatic amine groups, which makes chitin and chitosan suitable for typical reactions of amines. However, chitosan is chemically more active than chitin due to the presence of primary and secondary hydroxyl groups on each repeat unit, and the amine group on each deacetylated unit (Fig. 2). These reactive groups are readily subject to chemical modification to alter mechanical and physical properties of chitosan. The existence of amine groups in chitin and chitosan represents a great advantage because it enables distinctive biological functions as well as the application of modify- cation reactions. Excellent properties of these polysaccharides, such as biocompatibility, biodegradability, bioactivity, bioresorptivity, non-toxicity

and good adsorption properties make these materials very suitable and essential biomaterials and draw a great deal of industrial attention as probable alternatives to synthetic polymers.



Selection Criteria of Biopolymer for Scaffolding [6, 17].

The selection criteria include a certain feature such as chemical composition, molecular weight, solubility, shape and structure, hydrophilicity/hydrophobicity, surface energy, water absorption capacity, breakdown, and erosion mechanism. Polymer scaffolds are attracting a great deal of attention due to their unique features, such as the high surface-volume ratio, great porosity on the surface with a very small pore size, ability to control biodegradation, and mechanical properties. They have several biocompatibility benefits, versatility in surface chemistry, and biological properties that are important in tissue engineering application and organ replacement in regenerative medicine. In this chitosan has drawn a lot attention.

Porous chitosan scaffolds formation [8, 9, 10, 11].

Three dimensional highly porous scaffolds are fabricated by freezing and lyophilisation of chitosan solution. Some of the techniques used in scaffold formations are as follow.

i. **Freeze drying process**

During this process, the ice crystals are removed and 3D porous scaffolds with large surface to volume ratio are obtained that facilitates culture of large quantities of cell in small volume of scaffolds. The desired pore size is controlled by processing steps that include pre-freezing temperature, cooling rate, concentration and composition of polymer, and the geometry of thermal gradients during freezing.

ii. **Freeze Gelation Method**

In this method of chitosan scaffolding, chitosan is dissolved in 1M acetic acid to form a 2 wt. % polymer which is placed for frozen at -20 degree c. Frozen chitosan solution is than immersed in a pre-cooled NAOH/ethanol aq. solution at -20 ^{o}C to adjust its Ph allow gelation below freezing point of chitosan solution. Hence, this process of scaffolding is known as freeze gelation method.

iii. Freeze Extraction Method

In this method chitosan solution is prepared by dissolving chitosan (between 2 and 10% w/w) in water: acetic acid solution with ratio of 99:1, 97:3 & 95:5, these solutions are than stirred at 100 rpm and heated at 50 ^{o}C until they become homogenous. Then, they are poured in steel container with an internal diameter of 2 cm & height of 1cm and are phase separated at temperature of -20 ^{o}C for 24 hour. Subsequently, the sample are put in a bath of acetone at -20 ^{o}C for 24 hour to substitute water and then it was dried using SC-CO_2.

iv. **Solvent casting method**

A simple and most commonly used method for fabricating scaffolds for tissue engineering. This method involves mixing water soluble salt (e.g, NaCl) particles into a biodegradable polymer solution. The mixture is then cast into the mold of the desired shape. After the solvent is removed by evaporation or lyophilisation, the salt particles are leached out by water to obtain a porous structure. Solvent-Casting and Particulate Leaching technique can be applied to any polymer that is soluble in a solvent such as chloroform or methylene chloride.

v. **Gas Foaming Method**

Organic solvents residues left behind from the process of solvent -casting and particulate leaching can be toxic *in vitro* and elicit inflammatory responses *in vivo*. Gas -foaming process usually uses CO_2 as an agent for the pore formation. Solid polymer disks are exposed to high pressure CO_2 to allow saturation of CO_2 in the polymer. Thermodynamic instability is then created by reduction in pressure. This results in rapidly releasing CO_2 from the polymer, followed by nucleation and growth of gas bubbles (i.e, pores) in the polymer. The disadvantage is that it yields mostly a nonporous surface and closed -pore structure, with only 10 -30% of interconnected pores.

vi. **Phase separation method**

Thermally induced phase occurs when the temperature is decreased. Once the phase-separated system is stabilized, the solvent-rich phase is removed by sublimation leaving behind the polymer as a foam. Phase separation, which avoids harsh chemical or thermal environments, has been utilized to incorporate small bioactive molecules into scaffolds. One advantage of using phase separation is that the scaffolds often have good mechanical properties compared to salt- leaching technique.

Ideal characteristics that must contain a chitosan scaffold for use in tissue engineering and regenerative medicine [6, 7, 8, 11, 12].

Characteristics	Description of the characteristics
Biocompatibility	They must be accepted by host site and must not lead to rejection mechanism because of its presence.
Chemical stability	Chemical modifications not being present in a biological system implant or biodegradable in nontoxic products, at least during the scheduled time to regenerate tissue.
Chemically adequate surface	To have a chemically adequate surface for cell access, proliferation and cell differentiation.
Not to be toxic or carcinogenic	Its degradation products cannot cause local or systemic adverse effect on a biological system.
Absorbability and degradability	Absorbable, with controllable degradation and resorption rate to be the same as the *in vitro* and *in vivo* cell/tissue growth.
Adequate resistance and mechanical properties.	Resistance, mechanical properties, superficial characteristics, fatigue time, and weight, according to the receptor tissue is also required.
The proper design, size, and shape of the scaffolding.	Which allows having a structure with properties according to the needs of the receiving tissue to regenerate or repair.
Biodegradability	The biodegradable substitutes act as a temporary skeleton inserted into the defective sites of skeleton or lost bone sites, in order to support and stimulate bone tissue regeneration while they gradually degrade and are replaced by new bone tissue.
Bioactivity	Chitosan possess some biological properties like anti- microbial, anti-inflammatory, tissue inductive and conductive which play a synergistic role in tissue engineering
Bio-adhesive	The +ve charge of chitosan allows mucoadhesion with -vely charge mucus which result mechanical support to the scaly wounds.
Osteoconductivity	Encourage host bone adherence and growth into the scaffold.
Fabrication	Possess desired fabrication capabilities (e.g. being readily produced into irregular shapes of scaffolds that match the defects in the bone of individual patients.
Commercialisation	Be fabricated at an acceptable cost for commercialisation.

Tissue engineering applications of chitosan Skin [4, 11, 12, 13].

Wound healing is a biological process which involves growth and tissue regeneration. Chitosan matrix loaded bioactive components act together to re-establish the integrity of damaged tissue and replacement of lost tissue. Chitosan sponges are mainly used as a carrier for bioactive components and deliver continuous release of drugs for wound healing with the aid of soaking up the wound exudates and helping in tissue regeneration. Wound is frequently contaminated with a variety of bacteria, so the potential for infection is always present. This has encouraged the development of improved wound dressing that shows an antimicrobial effect through incorporation. Scaffold are intended to provide sufficient mechanical strength and stiffness to substitute initially for wound contraction forces and later remodelling of the tissue. It also protects from exposure of UV-radiation and present from darkening and thickening of scar- tissue and from permanent damage. Chitosan scaffold alone cannot initiate promising effect in wound healing process. The substantial development of composite material with chitosan network will provide effective role in tissue engineering. As proven, curcumin loaded chitosan nanoparticles impregnated into collagen-alginate scaffolds produce quick diabetic wound healing. Scaffold acts as 3D network with ECM and allow the free diffusion of water and bioactive molecules for soft tissue engineering application.

Bone [13, 14, 15, 16].

Composite materials are now playing predominant role as scaffolds in bone tissue engineering. For the repair of large bone defects, the mechanical and space-filling attributes of the scaffold are of primary importance. The biodegradable substitutes act as a temporary skeleton inserted into the defective sites of skeleton or lost bone sites. Basically, this new approach intended to improve the xenografts technique.

It is because the bioactive properties of xenografts are weaker, discomfort and long-lasting pain which are downside in comparative to tissue engineering. Hence, the main aim of tissue engineering is to eliminate the disadvantages of the clinical treatment associated with allografting and autografting. Chitosan 3D matrix provides three basic requirements like osteoinductive molecules, osteoconductive biomaterial scaffold and osteogenic cells. It also provides controlled long release of associated bioactive molecules to the site. In general, a tissue engineering process begins with the fabrication of a biologically compatible scaffold that will support living cells for their attachment, proliferation and differentiation, and thus promote tissue regeneration both *in vitro* and *in vivo.* Numerous chitosan composite study has been done to demonstrate the efficacy of new approach of preparing scaffold. The boundless research is taking place with the effort of making this technique more controlled, reliable and acceptable.

Conclusions

So far above fact is concerned, Tissue engineering is an ultimately ideal medical treatment for diseases that have been too difficult to be cured by existing methods. It acts as a promising tool in the field of biotechnology and medical engineering. Moreover, it is immensely acceptable in front of conventional xenograft process. Tissue scaffolds can not only cover the wound and provide a physical barrier, but can also offer a cellular skin with excretive biological components to stimulate re-epithelialization and formation of granulation tissue. Still there are many challenges that has to be faced during fabrication process and in selection of accurate polymer for the best choice of injured tissue. But boundless

of study is being carried out to solve these complications associated with the 3D matrix.

References

1. Ana Rita, Costa-Pinto, Rui L. Reis, Nuno M. Neves, Scaffold based bone tissue engineering: The Role of Chitosan, Tissue engineering: Part B. 2011, 17
2. Muschler GF, Negami S, Hyodo A, Gaisser D, Easley K, Kambic H. Evaluation of collagen ceramic composite graft materials in a spinal fusion model. Clin Orthop Relat Res, 1996; 328, 250.
3. Islam S, Bhuiyan Rahman MA, Islam MN, Chitin, Chitosan. Structure, Properties and Applications in Biomedical Engineering, Journal of Polymers and the Environment,1-13.
4. Zargar V, Asghari M, Dashti A. A Review on Chitin and Chitosan Polymers: Structure, Chemistry, Solubility, Derivatives and Applications, ChemBioEngRev 2015; 2: 1-24.
5. Majeti NV, Ravi Kumar. A review of chitin and chitosan applications, Reactive and Functional Polymers, 2000; 46:1-27.
6. Zahra J. Chitosan: A Brief Review on Structure and Tissue Engineering Application. J. of Applied Tissue Engineering Application. December. 2014; 1(1):3-7
7. Lin C-Y, Schek RM, Mistry AS, Shi X, Mikos AG, Krebsbach PH *et al.* Functional bone engineering using ex vivo gene therapy and topology-optimized, biodegradable polymer composite scaffolds. Tissue Eng.2005; 11(9–10):1589-2
8. Tingli Lu, Yuhui Li, Tao Chen. Techniques for fabrication and construction of three-dimensional scaffolds for tissue engineering, Int. J Nanomedicine. 2013; 8:337-35
9. Sughanthy Siva AP, Ansari MNM. A Review on Bone Scaffold Fabrication Methods, International Research Journal of Engineering and Technology. 2015; 2(6).
10. myweb.ncku.edu.tw/~jjhu/te_wk10.pdf.Scaffold Design and Fabrication.
11. Thein-Han WW, Kitiyanant Y, Misra RDK. Chitosan as scaffold matrix for tissue engineering, Materials Science and Technology, 2008, 24.
12. Zhong SP, Zhang YZ, Lim CT. Tissue scaffolds for skin wound healing and dermal reconstruction, Tissues scaffolds for skin wound healing, 2010, 2.
13. Martin Rodríguez-Vázquez, Brenda Vega-Ruiz, Rodrigo Ramos-Zúñiga, Daniel Alexander Saldaña-Koppel, and Luis Fernando Quiñones-Olvera, Chitosan and Its Potential Use as a Scaffold for Tissue Engineering in Regenerative Medicine, BioMed Research International, Article ID 821279, 15. 2015.
14. Venkatesan J, Kim SK. Chitosan Composites for Bone Tissue Engineering, Mar Drugs, 2010; 8(8):2252-2266.
15. LogithKumar R, KeshavNarayan A, Dhivya S, Chawla A, Saravanan S, Selvamurugan N. A review of chitosan and its derivatives in bone tissue engineering, Carbohydrate Polymers, 2016; 151:172-188.
16. Ana Rita Costa-Pinto, Rui L Reis, Nuno M Neves. Scaffolds Based Bone Tissue Engineering: The Role of Chitosan, tissue engineering: Part B, 2011, 17.
17. Dhandayuthapani B, Yoshida Y, Maekawa T, Kumar DS, Polymeric Scaffolds in Tissue Engineering Application: A Review, International Journal of Polymer Science, Article ID 290602. 2011.