1. Chitosan: A Biopolymer for Skin Regeneration

Review Article

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Abstract

Skin regeneration is a growing field of interest following the limited treatment modalities available for burn patients. An ideal treatment for burns is required to be fast, be able to restore complete functionality and be within the reach of patients. Such requirements may be achieved only by re-growth of the skin, but this is often limited in case of serious burns. The use of polymeric scaffolds presents a template for the regeneration of skin, allowing adherence of cells and providing support. Scaffolds of multiple materials have been tried; Chitosan scaffolds have yielded good results in various experimental aspects including biodegradability, low immunogenicity, compatibility, etc. Additionally properties such as angiogenesis, wound healing and induction of fibroblasts make it an ideal candidate for skin regeneration.

Keywords: Tissue engineering, skin regeneration, polymeric, scaffold, chitosan.

1. Role of Tissue Engineering

Tissue engineering is a promising therapeutic approach that involves combining living healthy cells of patients into three dimensional temporary scaffolds, made of natural and synthetic materials, in order to produce functional organs to be replaced back into the body. Despite many advances, tissue engineers still face significant challenges in repairing or replacing tissues that serve predominantly biomechanical functions. A major obstacle identified is, that the scaffold plays an important role as the extracellular matrix but is unable to create the correct microenvironment during the engineered tissue development to promote the accurate in vitro tissue development. [1, 2] Presently

in case of skin injury, the conventional solution is to use a skin substitute constructed using the concept of tissue engineering.

Serious injury to the skin, such as burns, trauma or chronic ulcers, requires immediate coverage to facilitate repair and restore skin function which can be done with skin grafts or tissue engineered skin.

2. Skin Substitutes & their Drawbacks [2]

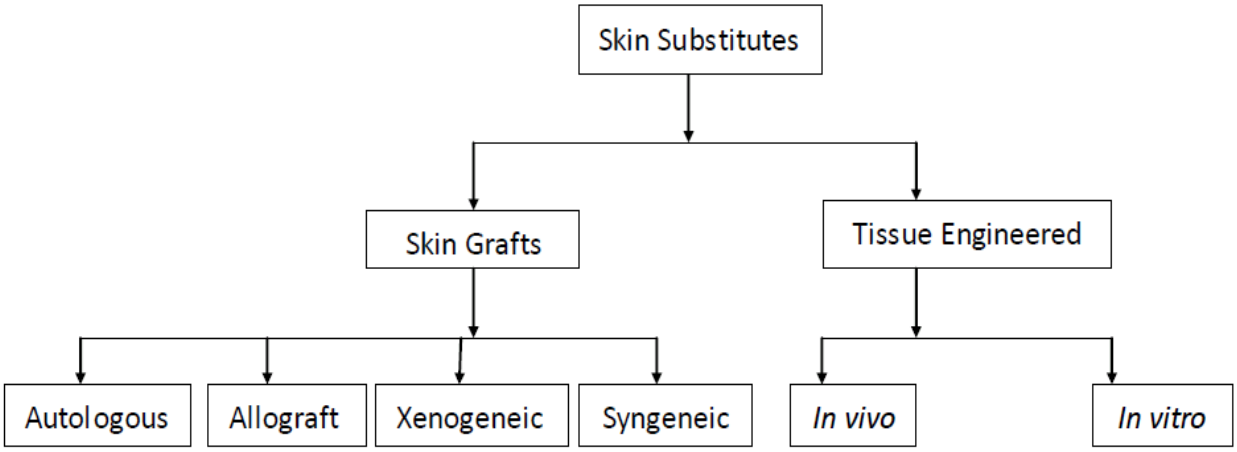

Figure 1: Classification of Skin Substitutes

Conventionally, tissue engineered skin exists as cells grown in vitro and subsequently seeded onto a scaffold or some porous material which is then placed in vivo at the site of injury. The gold standard for skin replacement still remains the autologous skin graft in which an area of suitable skin is separated from the tissue bed and transplanted to the recipient area on the same individual from which it must receive a new blood supply.

3. Problems with existing commercially available Skin Substitutes

- Reduced vascularization: Some existing skin substitutes do not allow angiogenesis to occur. This inability for substitutes to 'take' leads to cells in the replacement dying and ultimately the construct sloughs away from the host.
- Scarring: Scarring at the graft margins is problematic - functionally, mechanically and aesthetically. Scar tissue is not identical to

the tissue which it replaces and is usually of inferior functional quality. It is less resistant to ultraviolet radiation, and sweat glands, hair follicles do not grow back within scar tissue.

- Absence of differentiated structures: Bioengineered skin substitutes are often relatively simple single layered or bilayered structures. The absence of complexity with regard to differentiated structures means that presently available treatments offer none of the many other characteristics of functioning skin.
- Delay involved with cell culturing: Cells for the epidermal and dermal components can take between two and three weeks to expand to sufficient numbers for grafting purposes.
- Persistence of cells in heterologous grafts: Cell persistence may be desirable when covering a large area of missing skin but brings with it long-term safety challenges. E.g. Cell age may affect the duration that allogeneic cells survive in vivo.
- Biocompatibility, mechanical and handling properties: Currently available skin substitutes do not mimic normal skin composition or its mechanical properties. This is in part explained by the fact that the manufacturing processes employed are not sophisticated enough to recapitulate the developmental morphogenesis used to create skin naturally.
- Development, safety and product costs: The early stages of bringing a new tissue-engineered product to the market place can be costly.

4. Tissue Engineering Using Polymeric Scaffolds

The emerging and promising next generation of engineered tissues is relying on producing scaffolds with an informational function i.e. material containing growth factor sequences which facilitates cell attachment, proliferation and differentiation in vivo. A three-dimensional scaffold provides an extracellular matrix (ECM) analog which functions as a necessary template for host infiltration and a physical support to guide the proliferation and differentiation of cells into the functional tissues or organs[1,2], making the process faster and more suited to a particular individual.

4.1. Ideal Properties of Polymers to be used as a Scaffold for wound healing.

- The polymer should restore the epidermal barrier function and become incorporated into the healing wound.

- It should create an improved environment for epidermal regeneration and provide a barrier against infection and water loss.
- The material should be sufficiently permeable to water vapor and allow exudates to leave the wound.
- It would also be advantageous for the material to be haemostatic, transparent, and biodegradable as it restores normal function to the skin.

Some of the polymers currently used in tissue engineering are as follows:

Natural	Synthetic
Collagen	Polyesters
Gelatin	Poly(glycolic acid), poly(lactic acid) and their copolymers
Silk Fibroin	Polylactones
Fibrin	Poly(propylene fumarates)
Chitosan	Polyanhydrides
Starch	Tyrosine-derived polycarbonates
Alginate	Polyurethanes
Hyaluronan	Polyorthoesters

Table 1: Different types of Natural and Synthetic polymers used in tissue engineering

4.2. Natural Origin Polymers [2]

To date, no substitute or replacement for the patient's own skin has been prepared that shows qualities close to those of autologous grafts. Natural polymers have been found to be more suitable for tissue regeneration due to their similarity with normal body components. They have the ability to provide a microenvironment similar to the natural microenvironment and integrate well with growth factors. They perform a diverse set of functions in their native setting. For example, polysaccharides function in membranes and intracellular communication and also as storage, and proteins function as structural materials and catalysts. However, these natural origin polymers also have a few drawbacks.

Due to their similarity to biological substances, they often invoke an immunogenic response. There may be high degree of variability in natural materials derived from animal sources and they are structurally more complex than traditional materials; manipulation thus becomes more elaborate and complicated. They also tend to

degrade faster than the synthetic ones and they also have limited processing routes in many cases. Many different naturally derived polymers like collagen, hyaluronan, gelatin, etc. have been considered for use in tissue engineering with variable success, but the most striking and versatile polymer to date has been chitosan.

5. Chitosan

Chitosan is a cationic polymer obtained from chitin (A natural polysaccharide) comprising copolymers of β (1→4) glucosamine and N-acetyl-D-glucosamine. It is a derivative of chitin (poly-N-acetylglucosamine), which is the second most abundant biopolymer after Cellulose. [1]

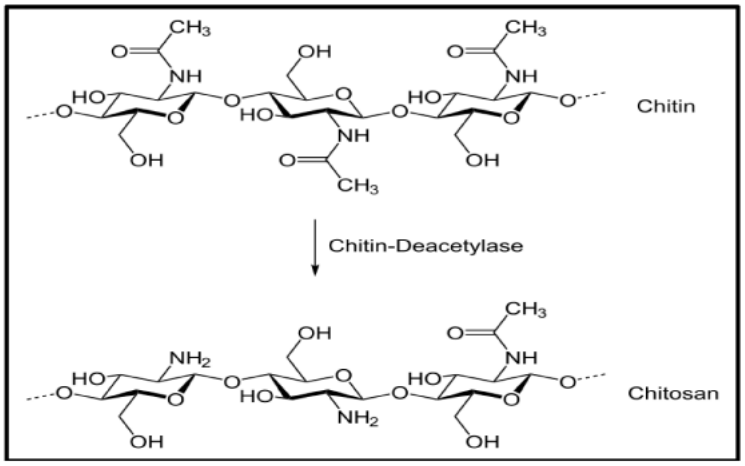

Figure 2: Conversion of chitin to chitosan

5.1 Properties of Chitosan crucial for wound healing

- Chitosan's property of binding with red blood cells allows it to rapidly clot blood. It has recently gained regulatory approval in the USA for use in bandages and other haemostatic agents.
- Chitosan modulates the functions of inflammatory cells and subsequently promotes granulation and organization.
- As a semi-permeable biological dressing, it maintains sterile wound exudates beneath a dry scab, preventing dehydration and contamination of the wound, to optimize conditions for healing.[4]
- It has been proved to be biologically renewable, biocompatible, non-antigenic, non-toxic and bio-functional.
- It is metabolized by certain human enzymes, especially lysozyme and thus can also be considered biodegradable.[1]
- It can act as an ideal wound dressing as it exhibits a positive charge, film-forming capacity, mild gelation characteristics and a strong tissue adhesive property.

- Chitosan induces fibroblasts to release interleukins, which are involved in migration and proliferation of fibroblasts.
- Chitosan based systems at micro and nano scales in combination with other polymers have been developed for skin tissue engineering, using electrospinning method and lyophilization.[1]

Chitosan has two major properties required for skin regeneration:

Antimicrobial property: Chitosan has been widely investigated as an antimicrobial agent due to its destabilizing effect on the outer membrane of gram-negative bacteria and permeabilization of the microbial plasma membrane by binding with sialic acid in phospholipids. [4]

Wound-healing effects of Chitosan: Chitosan and its derivatives can accelerate wound healing by enhancing the functions of inflammatory cells, such as polymorphonuclear leukocytes, macrophages, and fibroblasts or osteoblasts. [4]

6. Evaluation of Chitosan based scaffolds

6.1. Comparing the Biocompatibility of a Bilayer Chitosan Skin Regenerating Template, Human Skin Allograft, and Integra Implants in Rats [3]

A comparison by Shah Jumaat Mohd Yussof and his colleagues with regarding the biocompatibility of chitosan skin regenerating template with other skin substitutes i.e. Integra and Human skin allograft (HAS) for temporary coverage of clean burn wounds proved that, chitosan can physiologically stimulate the tissue repair process and favors angiogenesis. This evaluation provides the in vivo results of the angiogenic activity, inflammatory reactions and level of invagination by chitosan.

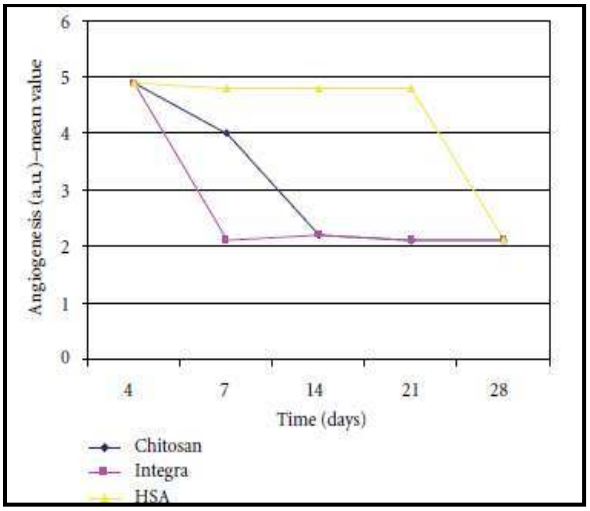

Figure 3: Level of angiogenesis v/s time. [3]

6.2 In vitro evaluation of a Biomedical Grade Bilayer Chitosan Porous Skin [5]

In this study by Chin Keong Lim et al chitosan porous skin regenerating template (CPSRT) was observed to support cell attachment. It was claimed that this growth

was likely due to the initial adaptation of cells to the 3D environment by adhering to the chitosan motifs in the construct. This study strengthens the possible application of chitosan as a template for skin regeneration providing an adequate microenvironment.

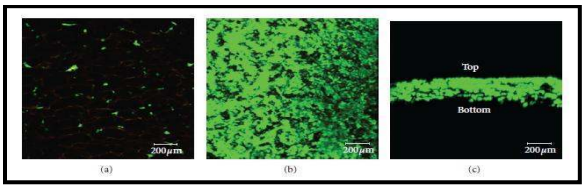

Figure 4: Live/dead cell staining of pHDF cultures seeded on CPSRTs. (a) Three-dimensional (3D) surfaces were scanned using a CLSM a day 5. (b) Viable pHDFs (green) were confluent at day 14. (c) Cross-sectional view of a CPSRT seeded with pHDF at day 14. [5]

6.3. Chitosan Nanofibrillar Scaffold for Skin Repair and Regeneration [6]

A Chitosan scaffold produced by accumulation of electrospun nanofibers was observed to be a better substrate for adhesion, growth, and differentiation of the three main skin cell types (keratinocytes, fibroblasts, and endothelial cells) than other types of chitosan device (films, sponges, gel, etc.). Moreover, they are fully biocompatible along with its progressive integration and colonization in vivo, in contrast with lamellar 3-D chitosan sponge that elicits the formation of a foreign body granuloma.

7. Modifications in the Chitosan Moiety

In order to realize the full potential of chitosan and bring a breakthrough in its utilization, there have been attempts made to modify chitosan to obtain various derivatives. [4] The probable methods are as follows:

- Chemical modification of chitosan molecules has been done at three reactive positions: the amino group at C-2 and the primary and secondary hydroxyl groups at C-3 and C-6, respectively [4]. This is of great significance because the fundamental skeleton of chitosan and its original outstanding physicochemical and biochemical properties as a biomaterial are retained after modification, while achieving desirable properties for tissue repair and regeneration applications.
- Cell Specific Ligands or Signaling Molecules have been introduced into chitosan in order to enable desirable interaction with cells and mediate specific cell responses and behavior. From the viewpoint of biomimetics, combination with extra-cellular matrix (ECM) proteins like collagen, fibronectin, and laminin is an attractive strategy for chitosan modification. However, direct modification by proteins

has been found to have several limitations due to possible immunogenicity, low cell-binding efficiency, and easy denaturation. These drawbacks can be overcome while maintaining the biofunctions by using small peptide sequences derived from the ECM.

- Cross linking is another effective approach for modulating characteristics of chitosan-based biomaterials for tissue repair. Typically, in the case of chitosan porous scaffolds, the mechanical strength and biostability can be enhanced by the cross linking treatment. Two types of cross linking are used for the chitosan porous scaffolds: Ionic cross linking and covalent cross linking.

7.1. Examples of Modified Chitosan

i. The hydrophobic character of the acetyl group on chitosan renders it water insoluble [7]. This water-insolubility of chitosan is disadvantageous for its wide application as an antibacterial agent. Hence to improve its water solubility, it is necessary to prepare anionic side chain- grafted, water-soluble chitosan (WSC) derivatives having zwitterionic properties [8]. To prepare these derivatives, mono (2-methacryloyl oxyethyl) acid phosphate and vinylsulfonic acid sodium salt have been grafted onto chitosan.

ii. Biopolymer blends between collagen and chitosan have been found to have potential to produce cell scaffolds with biocompatible properties since collagen is the major protein component of the extracellular matrix, providing support to connective tissues. [1] The chitosan – collagen blend showed promising properties including mechanical strength, biodegradability, and cell proliferation stimulating ability, which are crucial for tissue engineering. This biopolymer blend can further be cross linked with Glutaraldehyde in order to improve its biostability. [9]

iii. Due to the pH-sensitive character of chitosan, combination of chitosan with a thermo-responsive polymer, poly(N-isopropylacrylamide), has produced dual stimuli- responsive polymeric systems that can be used as delivery vehicles that respond to localized conditions of pH and temperature in the human body. [10]

iv. The poor solubility of chitosan at neutral and basic pH limits its biomedical applications, especially in physiological environments. Attempts have been made to boost the positive charge density, resulting in an enhanced solubility of chitosan over a broader range of pH [11]. Development of trimethyl chitosan (TMC) was an effort in

this regard. Chemical modification of chitosan to trimethyl chitosan provided derivatives that are soluble at neutral and basic pH.

8. Conclusion & Future Prospects

Chitosan has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic, hemocompatible and biofunctional. Due to these desirable properties, chitosan has been widely proposed as a scaffold material in tissue engineering applications and as a carrier for various drug delivery systems. Apart from the applications of the chitosan polymer itself, applications of chitosan in combination with other materials have also been studied. Such advances in the applications of chitosan are hinting towards the future of chitosan based scaffolds for skin regenerations. The low cost of chitosan in comparison with other biopolymers such as collagen is an incentive for further research and applications. Chitosan allows us to overcome the disadvantages of currently available skin substitutes and thus could soon replace them as a suitable, effective alternative providing a solution to thousands of burnt patients. It can be hypothesized from these findings that if the required in-vivo studies of chitosan are carried out, a fast-track solution to burns and scarring could be found, simultaneously enhancing the healing process of the body.

References

1. Malafaya P., Silva G., Reis R., 'Natural–origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications', Advanced Drug Delivery Reviews, 59(2007) 207-233.
2. Metcalfe A., Ferguson M., 'Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration', Journal of Royal Society, **4** (2007)413-437.
3. Yussof S., Halim A., Saad A., Jaafar H.,'Evaluation of the Biocompatibility of a Bilayer Chitosan Skin Regenerating Template, Human Allograft, and Integra Implants in Rats', ISRN Materials Science, **2011**(2011)1-7.
4. Dai T., Tanaka M., Huang Y., Hamblin M., 'Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects', NIH Public Access, **9** (7) (2011) 857-879.
5. Lim C., Halim A., Zainol I., Noorsal K., 'In Vitro Evaluation of a Biomedical-Grade Bilayer Chitosan Porous Skin Regenerating Template as a Potential Dermal Scaffold in

Skin Tissue Engineering', International Journal of Polymer Science,**2011**(2011)1-7.

6. Tchemtchoua V., Atanasova G., Aqil A., Filee P., Garbacki N., Vanhooteghem O., Deroanne C., Noel A., Jerome C., Nusgens B., Poumay Y., Colige A., 'Development of a Chitosan Nanofibrillar Scaffold for Skin Repair and Regeneration', Biomacromolecules,**12** (2011) 3194-3204.
7. Zang J., Xia W., Liu P., Cheng Q., Tahirou T., Gu W., Li B., 'Chitosan Modification and Pharmaceutical/Biomedical Applications', Marine Drugs, **8** (2010),1962-1987
8. Sashiwa H., Aiba S., 'Chemically modified chitin and chitosan as biomaterials', Progress in polymer science, **29** (2004)887-90.
9. Ma L., Gao C., Mao Z., Zhou J., Shen J., Hu X., Han C., 'Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering', Biomaterials **24** (2003) 4833-4841.
10. Alves N., Mano J., 'Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications', International Journal of Biological Macromolecules, **43**(2008)401-414**.**
11. Liu X., Ma L., Mao Z., Gao C., 'Chitosan-Based Biomaterials for Tissue Repair and Regeneration', Advance Polymer Science, **244** (2011) 81-128.