CHAPTER III

LITERATURE REVIEWS

3.1 Skin substitutes and wound healing

Supp, D. *et al.* [46]

This paper reviewed the structure and function of human skin and the available skin substitutes. There were several skin substitutes that had been useful for replacement or reconstruction of one or both layers of the skin, facilitating wound healing in several different clinical settings. These skin substitutes acted as temporary wound covers or permanent skin replacements, depending on their design and composition. The most basic of the skin substitutes were synthetic, acellular materials designed to act primarily as barriers to fluid loss and microbial contamination. One example that had been widely used for coverage of excised burn wounds was Integra. Integra Dermal Regeneration Template had proved to be particularly valuable in patients with large burns and limited autograft donor sites. Integra consisted of two layers. The dermal layer served as a matrix for infiltration by fibroblasts and other cells from the wound bed. As the collagen matrix was populated by these cells, it was gradually degraded and replaced by newly synthesized collagen. The silicone layer provided a functional barrier that was removed upon vascularization of the dermis, to be replaced by a thin layer of autograft. Other examples, such as Biobrane, Alloderm, Dermagraft etc., were also mentioned in this work.

Jones, I. *et al.* [47]

This review described the function of normal skin and a large number of new approaches and products that are emerging in the quest to develop biologically based skin substitutes for clinical use. Additionally, examination of the range of different products that are available and the current evidence for their effectiveness and their relative costs were shown. Integra, the most widely accepted synthetic skin substitute to be developed for use in burns by Yannas, was described in details. The pore size of

Integra has been designed at 70-200 μm in order to allow migration of the patient's own endothelial cells and fibroblasts. Smaller pores delayed biointegration, whereas larger pores provided an insufficient attachment area for invading host cells.

There were disadvantages to the use of this product. It was relatively expensive when compared with cadaveric allograft skin from skin banks, and the learning curve was reported to be steep, with high failure rates initially. The advantages were that it provided improved elasticity and cosmesis compared with an ultra-thin allograft, with reduced donor-site morbidity compared with a standard-thickness allograft, as it healed faster with less scarring. It avoided the risks of cross infection inherent with allografts. Integra had an important role in providing immediate wound cover following early excision in patients with insufficient autograft.

3.2 Scaffold fabrication

Kang, H. *et al.* [48]

In 1999, Hye-Won Kang *et al.* have studied a novel method for preparing porous materials. Biodegradable hydrogels were prepared through crosslinking of gelatin with glutaraldehyde in aqueous solution, followed by rinsing and washing. The porous scaffolds were obtained from freeze drying technique. For the process of freeze drying, water was homogeneously distributed into the hydrogel network, allowing the hydrogel network to uniformly enlarge and the ice to act as a porogen during the freezing process, and then sublimated the ice. The difference in pore size and inner structure reflected the different rates of heat transfer during the freezing process of the swollen hydrogels. A higher freezing temperature, the number of ice crystallization initially formed was lower, leading to an increased final size of ice crystals and the pore size of scaffold was increased. Furthermore, it was concluded that the present freeze drying procedure was a bio-clean method for formulating biodegradable sponges with different pore structures without use of any additives and organic solvents.

Mao, J. *et al.* [49]

In 2003, Jin Shu Mao *et al.* have studied on chitosan–gelatin polymer network scaffolds prepared via the freeze drying technique. Monolayer and bilayer scaffolds were obtained by using different pre-freezing methods. The monolayer scaffolds were prepared via place within a refrigerator at different temperature(-20°C, -40°C, -60°C), while bilayer scaffolds were prepared via contact with -56°C lyophilizing plate directly, then both were lyophilized. Microstructures, physical and mechanical and degradable properties of chitosan–gelatin scaffolds were studied.

The results showed that the bilayer scaffold was composed of a loose layer located on the border of air and a dense layer located on the border of freezing plate. The pore arrangement of bilayered scaffold was well regulated, while the pore configuration of monolayer scaffold was ruleless. In addition, changing the pre-freezing temperature and cooling rate would influence the mean pore size of the scaffold. The pore size became smaller and pore walls were thinner, while interconnectivity increased along with declining pre-freezing temperature. The scaffolds prepared from chitosan and gelatin could be utilized as a promising matrix for tissue engineering.

Pieper, J. *et al.* [50]

In this study, porous type I collagen matrices were prepared by freeze drying technique and crosslinked using dehydrothermal (DHT) treatment and/or 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC), in the presence and absence of chondroitin sulfate (CS). EDC covalently attached CS to collagen. DHT crosslinking is a physical method which was applied to stabilize collagen and to prevent matrix collapse while no cytotoxic reagents were introduced. Stabilization was believed to be due to the formation of interchain crosslinks as a result of condensation reactions either by etherification or amide formation. After DHT treatment, porous matrix morphologies were retained, as in non-treated matrices. DHT treatment of collagen resulted in a decrease in the free amine group content and in the water–binding capacity, and an increase in the tensile strength. However, this treatment did not significantly contribute to matrix stabilization since DHT-treated and non-treated

matrices had comparable denaturation temperatures and were degraded by collagenase and pronase to a similar extent.

Ozeki, M. *et al.* [23]

In 2004, Makoto Ozeki and Yasuhiko Tabata have studied the *in vivo* degradation of gelatin hydrogels in terms of various crosslinking methods. Various hydrogels were prepared from different gelatins, extracted from different sources, through four types of crosslinking methods, which were glutaraldehyde treatment, dehydrothermal treatment, ultraviolet irradiation and electron beam irradiation. Two measurements to evaluate the extent of hydrogel crosslinking, which were water content and theory of rubber elasticity, were performed. Following subcutaneous implantation of ^{125}I-labeled gelatin hydrogels into mice, the radioactive remaining was measured at different time intervals to assess the *in vivo* degradability of hydrogels.

The results showed that the number of crosslinks per gelatin molecule increased with increasing time of glutaraldehyde and dehydrothermal treatments, irrespective to the gelatin type. The number of crosslinks of gelatin hydrogels increased with UV irradiation time. For electron beam irradiation crosslinking, the number of crosslinks tended to increase as the irradiation dose increased. In addition, when the *in vivo* biodegradability of hydrogels was compared by the measurement of hydrogel water content, great dependence of the gelatin type and crosslinking method on the degradation was observed. The study indicated that the biodegradability of gelatin hydrogels could be determined by the number of crosslinks per gelatin molecule from the theory of rubber elasticity, irrespective to the gelatin type and crosslinking method.

3.3 Properties of scaffolds

Choi, Y. *et al.* [51]

In 1999, Young Seon Choi *et al.* have studied on an absorbable sponge which is composed of gelatin and alginate. The sponge was prepared by crosslinking with

EDC-containing acetone:water mixture and the crosslinking degree was characterized by trinitrobenzenesulfonic acid (TNBS) assay. In addition, the sponge was tested for water uptake ability, *in vitro* drug release and collagenase degradation to confirm the applicability as a wound dressing material. The results showed that the porosity of sponge increased with increasing the alginate content in the sponge, resulting in enhanced water uptake ability. The results from *in vitro* tests showed that sponge loaded with silver sulfadiazine (AgSD) or gentamicin sulfate (GS) slowly released drugs for up to four days and the crosslinked sponge resisted *in vitro* collagenase digestion for up to three days. For an *in vivo* animal test, a full-thickness wound was covered with the gelatin-alginate sponges with or without AgSD and a vaseline gause as a control. The sponge containing AgSD showed better wound healing effect in view of a rapid proliferation of fibroblast and epidermal cells in the wound of a rat and a significant reduction of infectious cells after 12 days, compared with the sponge without drugs and the vaseline gauze.

Mao, J. *et al.* [52]

In 2003, Jin Shu Mao *et al.* have studied on the properties of chitosan–gelatin scaffolds, which were modified by incorporation of hyaluronic acid in the surface or bulk phase through crosslinking with N,N (3 dimethylamino-propyl)-N'-ethyl carbodiimide (EDC) and N-hydroxysuccinimide (NHS) in 2-morpholinoethane sulfonic acid (MES) buffer. The corresponding scaffolds were prepared through freeze drying.

The comparative study on properties of surface modified scaffold and polyblend scaffold revealed that surface modified scaffold was characterized by its smaller pore where a fiber texture could be found. The addition of hyaluronic acid led to an enhancement in water adsorption, elongation at break and fibroblasts adhesion, while slowing down the rate of biodegradation of chitosan–gelatin scaffold. The result from *in vitro* degradability showed that the rate of weight loss of surface modified scaffold was significantly quicker than that of polyblend scaffold because the smaller pore increased surface area. Human fibroblasts adhered and proliferated well on the scaffolds *in vitro*.

Bigi, A. *et al.* [53]

The glutaraldehyde (GTA) crosslinked type A gelatin films have been investigated for the mechanical, thermal, swelling, and release properties. Air-dried films were submitted to treatment with GTA solutions at concentrations ranging from 0.05 to 2.5wt%. The crosslinking degree, determined by TNBS assay, amounted to about 60% at the lowest GTA concentration and increased up to values near 100% at GTA concentrations ≥ 1wt%. Simultaneously, the deformability of the films decreased, whereas the stress at break and the Young's modulus increased. A crosslinking degree of about 85%, obtained using 0.25wt% GTA, was enough to prevent gelatin release in buffer solution and to provoke a significant reduction of the swelling in physiological solution. Furthermore, crosslinking greatly affected the thermal stability of the samples, indicated by the results of differential scanning calorimetry (DSC) carried out on wet and air-dried films. The data suggested that the use of GTA at low concentration, which was desiderable to prevent toxicity, allowed to modulate the physico-chemical properties of gelatin films, in order to obtain stable materials with a wide range of possible biomedical applications.

Lee, S. *et al.* [54]

Porous gelatin scaffolds were prepared using a salt-leaching method and these were compared to scaffolds fabricated using a freeze drying method. The results showed that the freeze dried gelatin scaffolds appeared in the interconnected network pore configuration, a membrane-like structure. The structure of the vertical channels was uniformly distributed, and formed an interconnected pore structure. Exceptionally, the cross-sectional morphology of low gelatin concentration scaffold formed a web-like structure. A cross-sectional structure of a scaffold prepared using the salt-leaching method exhibited interconnected pore structures, but did not form a membrane-like structure between pores. The initial modulus of the scaffolds increased with decreasing NaCl content in the salt-leached scaffold. In the freeze dried scaffolds, the mechanical strength increased with increasing gelatin content of the solution. In the enzymatic degradation studies, the degradation rates of the scaffolds prepared using the salt-leaching method were slower than those of the scaffolds

prepared using the freeze drying method. After 1 week of *in vitro* culturing, the fibroblasts in salt-leached scaffolds were mainly attached on the surface of the pores in the scaffold, whereas cells seeded on freeze dried scaffolds were widely distributed and aggregated on the top and the bottom of the scaffold. An *in vivo* study of cultured artificial dermal substitutes after 1 week showed that an artificial dermis containing the fibroblasts enhanced the re-epithelialization of a full-thickness skin defect when compared to an acellular scaffold.

Dawlee, S. *et al.* [55]

They have studied on the gelatin hydrogels prepared from chondroitin sulfate with different degrees of oxidation. They were characterized for degree of crosslinking, equilibrium swelling, water vapor transmission rate, internal structure, and blood-compatibility. Degree of crosslinking of the gels determined by TNBS assay showed that, higher the degree of oxidation of the polysaccharide, higher the degree of crosslinking. Examination of the internal structure by SEM showed that the hydrogels were highly porous in nature with interconnected pores ranging from 50 to 200 μm. Equilibrium swelling showed that the gels retained about 90% of water and did not undergo dehydration rapidly. Furthermore the hydrogels were non-toxic and blood-compatible.

Angele, P. *et al.* [56]

The study investigated the species-related differences of collagen scaffolds with and without 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) /N-hydroxysuccinimide (NHS) -crosslinking. Resistance against collagenase digestion, swelling ratio, amino acid sequence, shrinkage temperature, ultrastructural matrix morphology, crosslinking density and stress–strain characteristics were determined to evaluate the physico-chemical properties of equine- and bovine-collagen-based scaffolds. The results revealed a highly significant effect of collagen type, crosslinking and time on degradation of the collagen samples by collagenase treatment. Crosslinked equine collagen samples showed a significantly reduced swelling ratio compared to bovine collagen samples. The amino acid composition of

equine collagen revealed a higher amount of hydroxylysine and lysine. Shrinkage temperatures of non-crosslinked samples showed a significant difference between equine (60°C) and bovine collagen (57°C). Moreover, the analysis revealed a highly significant effect of collagen type, crosslinking and matrix condition on rupture strength measured by stress–strain analysis. The ultrastructure, the crosslinking density and the strain at rupture between collagen matrices of both species showed no significant differences. For tissue engineering purposes, the higher enzymatic stability, the higher form stability, as well as the lower risk of transmissible disease made the case for considering equine-based collagen. This study also indicated that results obtained for scaffolds based on a certain collagen species may not be transferable to scaffolds based on another, because of the differing physico-chemical properties.

Bigi, A. *et al.* [57]

This work reported a study on the influence of the renaturation level of gelatin on the mechanical and swelling properties of gelatin films. Films at different renaturation level were obtained from gelatin samples with different Bloom strength. It was verified that the triple helix content, calculated from the values of the enthalpy of denaturation associated to the endothermal transition at about 41°C of gelatin, increased with Bloom strength. The DSC results were further supported by the results of the X-ray diffraction carried out on the same samples. The increase of triple helix content provoked a significant reduction in the degree of swelling, and a remarkable improvement of the mechanical properties of the films. The elastic Young's modulus increased linearly with the renaturation level, from 3.6 to 12.0 MPa. Crosslinking with 1wt% of GTA remarkably reduced the degree of swelling of all the samples, and induced a further increase in the Young's modulus, which reached values up to 27 MPa.