CHAPTER 6

SUMMARY AND CONCLUSION

6.1 SUMMARY

6.1.1 Type I Collagen and its Daughter Peptides for Targeting Mucosal Healing in Ulcerative Colitis: A New Treatment Strategy

i) The therapeutic efficiency of intra-rectally delivered collagen and collagen hydrolysate was compared to that of mesalamine which served as the positive control, while the vehicle acted as negative control.

ii) The three clinical assessment parameters, rectal bleeding, stool consistency and loss in body weight, indicated an improvement in the disease condition with collagen, collagen hydrolysate and mesalamine treatment when compared to the negative control.

iii) The clinical activity index, a measure of the overall severity of the disease, showed that collagen hydrolysate gave better overall recovery than collagen and mesalamine.

iv) Among the groups that underwent therapy, collagen hydrolysate showed maximum downregulation of VEGF followed by mesalamine and collagen.
v) Both collagen and collagen hydrolysate therapy can effectively counter the major inflammatory markers (TNF-α, IL-1β and IL-6) involved in UC, and at the same time might also be able to provide protection to the intestinal mucosa from MMP action.

vi) Interestingly, a significant reduction in mucosal damage and faster regeneration of damaged mucosa seen with short term treatment of collagen and collagen hydrolysate and is consistent with the rectal bleeding scores.

vii) The efficacy of collagen and collagen hydrolysate in aiding mucosal regeneration is established. Figure 6.1 shows the graphical representation of the hypothesis on the effect of collagen and its hydrolyzed peptides on the healing of mucosal ulcers in ulcerative colitis.

![Figure 6.1](image)

**Figure 6.1** Graphical representation of the hypothesis on the effect of collagen and its hydrolyzed peptides on the healing of mucosal ulcers in ulcerative colitis
6.1.2 Preparation and Evaluation of Mesalamine Collagen *In Situ* Rectal Gel: A Novel Therapeutic Approach for Treating Ulcerative Colitis

i) In ulcerative colitis topical therapy of using mesalamine reduces systemic exposure. Gelation property of collagen had been strategized for the topical delivery of mesalamine in a controlled manner.

ii) Developed collagen-mesalamine undergoes sol-gel transition under physiological pH and temperature which was confirmed by rheological studies.

iii) The *in vitro* release profile demonstrated sustained release of mesalamine over a period of 12 h through matrix diffusion controlled mechanism.

iv) In the in-vivo study, mesalamine collagen *in situ* gel treated test group showed faster regeneration of mucosal damage and significant reduction in the rectal bleeding score. The presence of collagen had served as a matrix for damaged mucosa and aided in faster regeneration of diseased mucosa.

v) Immunohistochemical analysis revealed CD 34 expression was increased with collagen mesalamine in situ gel treatment compared to the mesalamine and the control group.

vi) Among the groups that underwent therapy, collagen mesalamine in-situ gel showed marked reduction in the TNF-α and VEGF expression compared to mesalamine and the control group.
vii) Collagen-mesalamine *in situ* hydrogel had been an effective strategy for the treatment of ulcerative colitis (Figure 6.2).

**Figure 6.2** Graphical representation of the treatment strategy of using mesalamine collagen *in situ* rectal gel for ulcerative colitis

6.1.3 Sol-Gel Assisted Fabrication of Collagen Hydrolysate Composite Scaffold: A Novel Therapeutic Alternative to the Traditional Collagen Scaffold

i) Sol-gel transition methodology was adopted to develop the collagen hydrolysate composite scaffolds (CHCS), and the effectiveness of the CHCS was investigated against the traditionally used collagen scaffolds for wound healing therapy.
ii) Collagen hydrolysate scaffold was prepared using the acid/base catalyzed sol-gel transition of a silica precursor, tetraethoxysilane.

iii) The scaffold with the final composition of Collagen hydrolysate: Chitosan: TEOS (1:0.3:1.5%, w/v) resulted in good foam stability and uniform spongy appearance.

iv) CHCS exhibits porous morphology with pore sizes varying between 380 to 780 µm. Incorporation of silica conferred CHCS with controlled biodegradation and better water uptake capacity.

v) The cell proliferation with CHCS treatment is significantly higher than that of collagen and chitosan-silica treatment.

vi) CHCS exhibits moderate antimicrobial activity against two gram positive strains, *Staphylococcus aureus* and *Bacillus subtilis*, and one gram negative strain *Escherichia coli*. The antimicrobial activity of collagen hydrolysate is a great value addition for CHCS in wound care therapy.

vii) In *in vivo* efficacy studies, the wounds dressed with CHCS healed faster than those dressed with collagen scaffold. Figure 6.3 shows the graphical representation of the fabrication of collagen hydrolysate composite scaffold for wound healing applications.

viii) Notably, CHCS provides faster healing in comparison to some of the commercially successful collagen composite scaffolds viz., BIOSTEP and FIBROCOL PLUS. Hence CHCS could certainly be an effective material for advanced wound care treatment.
6.1.4 Clinical Study to Evaluate the efficacy of Collagen Hydrolysate Composite Scaffold for the Treatment of Chronic Wounds in Leprosy Afflicted Patients

i) The effectiveness of collagen hydrolysate composite scaffold in treating chronic venous leg ulcers of leprosy afflicted patients.

ii) All 5 patients who received treatment using CHCS showed good progress in ulcer healing

iii) There were no adverse reactions reported and all patients expressed satisfaction in the treatment outcomes.
6.2 CONCLUSION

Type I collagen and its daughter peptides called collagen hydrolysate (CH) are hypothesized as healing matrices to target the recuperation of internal mucosal ulceration. Treatment with collagen and collagen hydrolysate are as effective, and in some aspects even more effective, than mesalamine in countering the pathological mechanisms of UC. Both collagen and collagen hydrolysate held a significant advantage over mesalamine in reducing the rectal bleeding, which is an indirect measure of mucosal healing. More importantly, the expression levels of key molecular factors shows that collagen and collagen hydrolysate have a role that is greater than just regeneration. Therefore, both collagen and collagen hydrolysate can be considered as potential candidates for comprehensive therapy for UC by targeting mucosal healing in addition to the existing therapy which targets inflammation. Currently, Mesalamine remains the first line drug for treating mild to moderate UC. The administration of collagen, a healing protein with the mesalamine therapy acted as a synergistic combination for effective management of UC. Interestingly, the histological score for mucosal damage and clinical score for rectal bleeding was significantly reduced for collagen-mesalamine in situ gel in comparison to mesalamine suspension. This therapeutic strategy of utilizing the sol–gel behaviour of collagen in enhancing the availability of mesalamine for longer period and synergistic efficacy of collagen and mesalamine combination had augured very well for the treatment of UC.

Collagen is one of the most widely used biomaterial for advanced wound care management. Collagen hydrolysate composite scaffold (CHCS) was fabricated with sol-gel transition procedure using tetraethoxysilane as the silica precursor. Incorporation of silica conferred CHCS with controlled biodegradation and better water uptake capacity. Notably, 3T3 fibroblast
proliferation was seen to be significantly better under CHCS treatment when compared to treatment with collagen scaffold. The antimicrobial activity of collagen hydrolysate is a great value addition for CHCS in wound care therapy. *In vivo* preclinical experiments with full thickness excision wounds in rat model demonstrated that wounds treated with CHCS showed accelerated healing when compared to wounds treated with commercially available collagen composite scaffolds. In clinical study, CHCS has proven to be an effective biomaterial even for ulcers which had wound history of many years that had been previously subjected to a variety of treatment regimens without much success. Thus, CHCS a first of its kind material that can be considered as a promising treatment option for chronic ulcers that have been non-responsive to conventional treatment.