Highly Purified Atelopeptide Human Placental Collagen

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Introduction:
Collagen is the most abundant protein in connective tissues in mammals and has a long history of medical and surgical application in humans. However, many collagen-based medical products are of bovine origin, which have the potential of causing immunologic responses in patients. Human collagen offers the significant advantage of low immunogenicity. This abstract presents a process to isolate high purity, atelopeptide collagen from human placenta.

Methods:

Donor Eligibility and Screening: Each placenta donor was carefully screened using comprehensive medical and social histories. Tissues were procured, processed, and tested in accordance with standards established by the AABB and the FDA (21 CFR 1271). Infectious disease testing was performed at a CLIA-certified laboratory.

Environmental and Process Controls: Processing was conducted in Class 100-1000 cleanrooms using aseptic techniques, sterile chemicals and equipment to minimize potential risk of biological contamination and disease transmission to recipients.

Extraction Process: A number of frozen placentas that individually passed donor eligibility were thawed and ground into small particles, followed by extensive washing steps to facilitate removal of cells, cellular and blood components. Collagen in the resulting tissue was then solublized with pepsin under acidic conditions using optimized process parameters, followed by micro-filtrations to remove un-solublized tissues. The filtered solution was further purified and recovered via collagen precipitation from a sodium chloride solution. The purified collagen solution was then subject to an ultrafiltration step for salt-removal and concentration. The final solution contained approximately 3 mg collagen/ml of 10 mM hydrochloric acid solution and was filtered through a 0.2 μm sterile filter to achieve sterility.

Results:
Under optimized process conditions, the atelopeptide collagen product shows high purity demonstrated in SDS-PAGE analysis (Figure 1), and high Type I content analyzed in ELISA assays (Table 1). The process was reproducible at pilot scale and yielded 4 – 5 g of high quality collagen per placenta (average), analyzed by hydroxyproline assay.

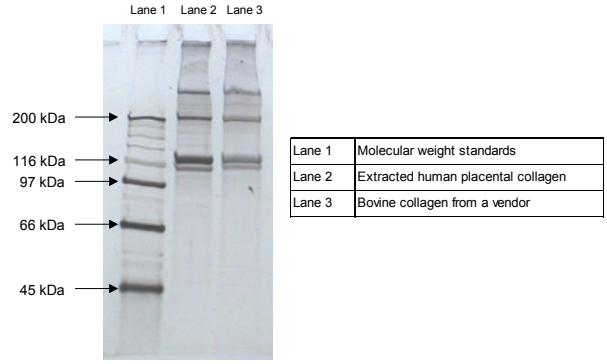

Figure 1. Extracted human placental collagen purity by SDS-PAGE analysis

	Extracted, Atelopeptide Human Placental Collagen Product
Collagen Type I	~ 95%
Collagen Type III	~ 3 %
Collagen Type IV	< 2 %
Extraction Yield	4 - 5 g collagen /placenta
Steriltiy	Sterile, UPS
Endotoxin Content	< 0.05 EU/ml, UPS

Table 1. Extracted human placental collagen type purity and extraction yield

Discussion:
Although the isolation of collagen is well documented in the literature, our optimized process is simple, scalable and generates high purity collagen solution from a complex source, the human placenta. This purified collagen solution can be formulated into various configurations for medical, surgical, pharmaceutical and cell therapeutic applications. Further more, the low content of Type IV collagen in the product significantly reduces the immunogenic risk. The high yield may directly lead to low manufacturing cost of this valuable biomaterial.