Effect of collagen sources on the in-vitro performance of collagen implant

Chamika K. Madawala[1],Priyanka Srinivasan[1], Ali Muhammad[1], Seo Youngee[2] , Maxwell Korang-Yeboah[1], Xiaoming Xu[1], Muhammad Ashraf [1], Yan Wang[3], Bin Qin[3], Qiangnan Zhang[3] , Nahid S. Kamal*[1]

1 Office of Pharmaceutical Quality (OPQ)/Office of Pharmaceutical Quality Research, CDER/FDA ;2 OPQ/Office of Product Quality Assessment I, CDER/FDA;
3 Office of Generic Drugs/Office of Research and Standards, CDER/FDA

Presenting Author*

U.S. FOOD & DRUG ADMINISTRATION

Fall 2024 SBIA Workshop

POC: Nahid Kamal, OPQ/OPQR V

Email: nahid.kamal@fda.hhs.gov

Disclaimer: This poster reflects the views of the authors and should not be construed to represent FDA's views or policies.

PURPOSE

The Bupivacaine HCl collagen implant is a matrix-type drug delivery system used for managing post-surgical pain. This implant's matrix comprises of purified Type I collagen as the sole excipient. The source, extraction method, and processing conditions for Type I collagen may vary, leading to differences in its physical and chemical properties. These variations can potentially impact the quality, drug release characteristics, and in vitro performance of the collagen implant. Hence, the objective of this study is to investigate how different sources of collagen affect the quality parameters (morphology, porosity, drug localization) and the performance (in vitro drug release) of the Bupivacaine collagen implant.

METHODS

Purified Type I collagen were characterized after obtaining from three sources (A, B and C). The nitrogen to hydroxyproline ratio (N/Hyp) were evaluated; solid-state characteristics were tested by differential scanning calorimetry (DSC) and X-ray diffraction (XRD). Bupivacaine HCl solution and collagen solution were prepared separately in acidified water (pH 4.5) at 38°C ± 2°C. The two solutions were mixed using a high shear homogenizer to prepare the final collagen dispersion. The homogenizer speed was set at 2000 rpm and mixed for 15 mins. The homogenizer speed and mixing time were predetermined for adequacy. The collagen dispersion was mixed again for 15 mins and filtered through a 250 µm nylon filter. The resulting dispersion was then further characterized for viscosity and morphological parameter evaluation. The dispersion was filled in polyethylene glycol terephthalate containers and lyophilized using optimized condition. The manufacturing process, and characterization of raw collagen, collagen dispersion and of final implants are provided in the schematic below.

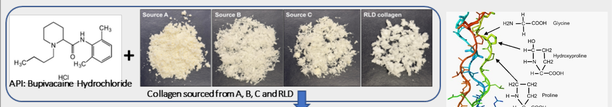

Figure 1: Schematic representation of manufacturing process and characterizations

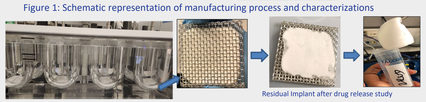

Figure 4: Drug release study using USP type II apparatus and custom-made sinkers.

Figure 2: Triple helix structure (left) and critical amino acids (right) of collagen

Figure 3: HPLC to quantify drug

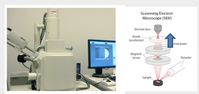

Figure 5: SEM for morphological analysis

Table 1: IVRT experimental conditions

Apparatus	USP2 w/ steel sinker
Rotation speed	50rpm
Medium	Phosphate buffer pH6.8
Temperature	37±0.5 °C
Sampling time	0.5,1,2,3,4,5, 6 hours

RESULTS & DISCUSSIONS

Characterization results of raw collagen

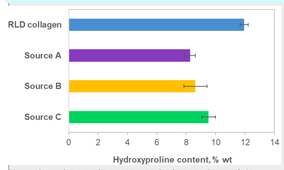

Figure 6 . Hydroxyproline assay results (mean ±SD, n=4) in various test collagen varied with no significant (p>0.05) differences.

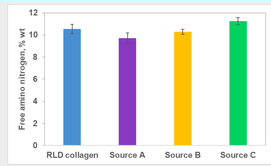

Figure 7. Free amino nitrogen assay (mean ±SD, n=4) revealed all three collagen samples showed similar free amino nitrogen profile including RLD, no significant differences (p>0.05).

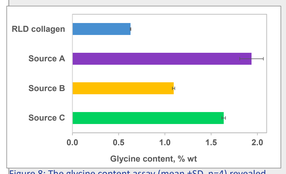

Figure 8: The glycine content assay (mean ±SD, n=4) revealed the value varied with significant differences (p<0.05).

Table 2. Nitrogen to Hydroxy Proline (N/Hyp) ratio (mean ±SD, n=4) , results were comparable (p>0.05)

Collagen source	N/Hyp ratio
RLD collagen	0.89 ± 0.05
A	1.18 ± 0.08
B	1.18 ± 0.16
C	1.19 ± 0.01

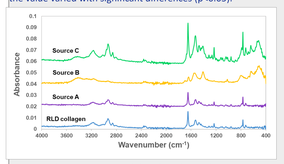

Figure 9: The FTIR spectra exhibited similar peak patterns regardless of sources of collagen.

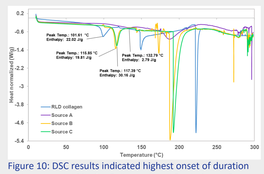

Figure 10: DSC results indicated highest onset of duration and greater stability for Collagen A and B relative to C and RLD collagen.

Characterization results of lyophilized implants

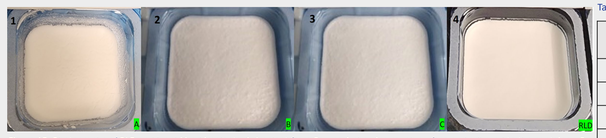

Figure 15: Photographs of RLD and lyophilized implants. 1: Implant from Source A showing rough surface and adhered to the container; 2 and 3: Implant from Source B and C showing smooth surfaces and were not sticking to the containers; 4: RLD implant.

Table 3: Total Porosity % of each implant.

Implant Source	Total porosity % (3D analysis)
RLD	86.87
Source A	95.22
Source B	92.41
Source C	94.62

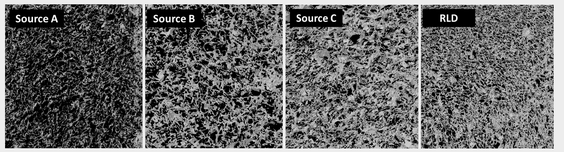

Figure 17: Micro-CT images showing the porous nature of the implants from source A, B, C and RLD.

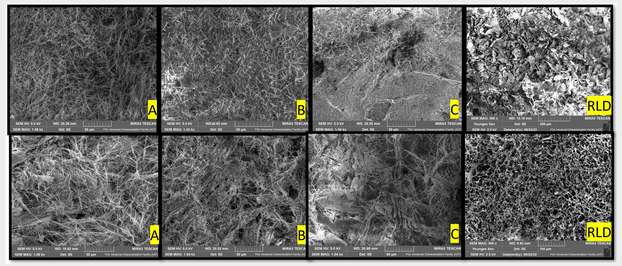

Figure 16: Surface morphology (upper tier) and cross sectional (lower tier). The topography and cross-sectional area of these implants revealing a randomly interconnected fibrous structure with pores. However, the density of this interconnected fibrous structure was different for all the implants and not comparable to RLD. On the other hand, RLD displayed a prominent honeycomb-like structure different than that of Implants A, B, and C.

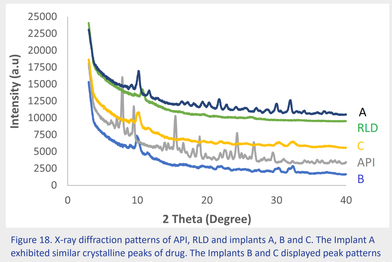

Figure 18. X-ray diffraction patterns of API, RLD and implants A, B and C. The Implant A exhibited similar crystalline peaks of drug. The Implants B and C displayed peak patterns similar to that of RLD along with few smaller peaks suggesting a semicrystalline nature of the drug.

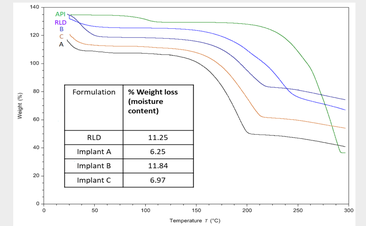

Figure 19. The moisture content results of Implant B and RLD were similar. The Implant A and C contained significantly lower moisture levels.

Figure 20: Filtrate collected after filtration to measure drug loss.

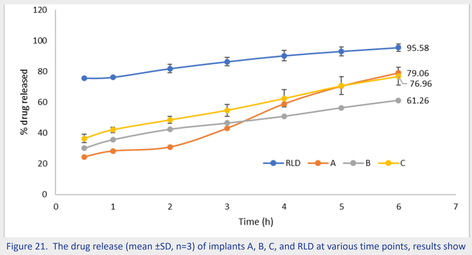

Figure 21. The drug release (mean ±SD, n=3) of implants A, B, C, and RLD at various time points, results show that 79%, 61% and 77% of the drug were released from implants A, B, and C, respectively, compared to the reference listed drug (RLD) at 96%.

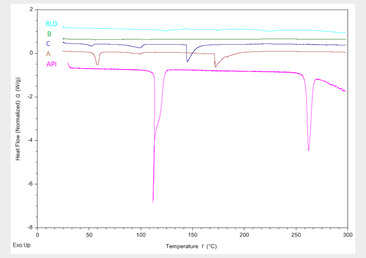

Figure 22. The DSC results indicate that Implant B and RLD contain drug in amorphous form, while the Implants A and C contain drug in crystalline or semicrystalline form.

Characterization results of dispersions

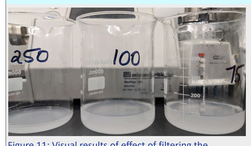

Figure 11: Visual results of effect of filtering the dispersions using different filter bags sizes (75µ,100µ and 250µ). No differences observed despite having different pore sizes of the bags.

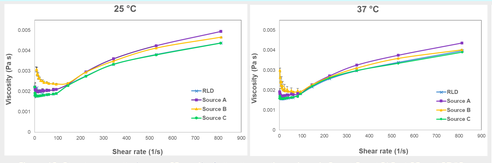

Figure 12: Rheology results (mean ±SD, n=4) of all collagen dispersions A, B and C and RLD at 25 and 37 °C exhibit a low viscosity (exhibiting Non-Newtonian flow), with shear thickening effect.

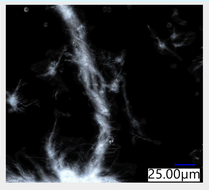

Figure 13: Representative digital optical microscopic image of the fibrillar structure of collagen sources A-C.

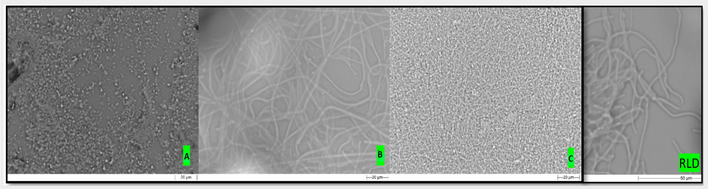

Figure 14 (image below): MDRS images of collagen dispersions A, B & C without drug and RLD dispersion: (a) loose clustered micro fibrillar structure of collagen dispersion A, (b, c) large interconnected tangled fibrillar structure of collagen dispersions B and C, (d) RLD. The fibrillar structure was not affected by changing mixing speed and mixing time.

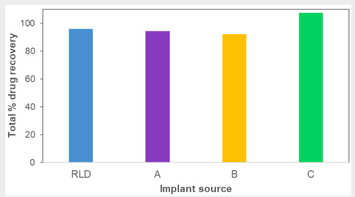

Figure 23: The drug recovery from residual implants at the end of drug release experiments, At the end of 6-hour study period, 26-27% drug remained unreleased in implants B and C compared to 2% in implant A and 0.01% in RLD.

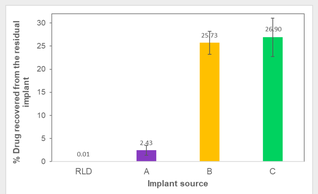

Figure 24: Total % drug recovered from the Implants (mean ±SD, n=6) after mass balance study.

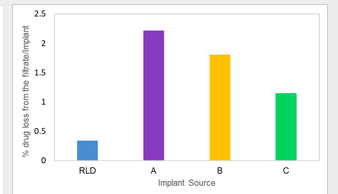

Figure 25: Drug loss from the filtrate, about 1-2% of the drug per implant was recovered from the filter before lyophilization.

CONCLUSIONS

The structural properties of collagen from various sources and the morphology and microstructure of the resulting implants appeared to be similar with different fibril structures which may explain the differences in the preliminary drug release and in drug loss during filtration. Further studies to comprehensively examine the mechanism of drug release, binding with different collagens, localization of drug in the implants and the overall influence of different sources of these collagens on the quality and performance of implants are in progress.

FUNDING

This project is supported in part by appointing ORISE fellows (Youngee Seo, Priyanka Srinivasan, Chamika K. Madawala) to the Research Participation Program at the U.S. FDA by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.