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

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PURPOSE

The Bupivacaine HCl collagen implant is a matrix-type drug delivery system used for managing post-surgical pain. This implant's matrix comprises 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 obtained from three sources (A, B and C). The hydroxyproline to nitrogen 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 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 shown in the schematic below.

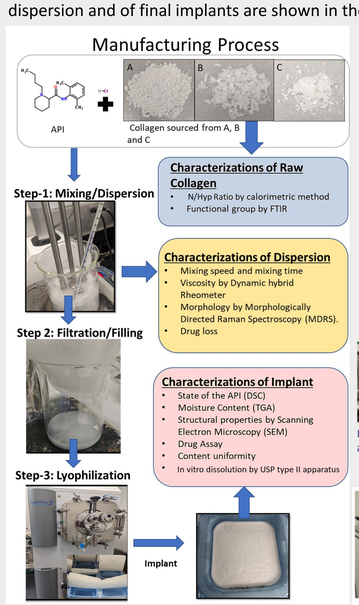


Figure 1: Schematic representation of manufacturing process and characterization

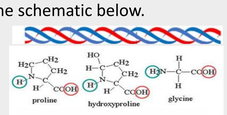


Figure 2: Triple helix structure (top) and critical amino acids (bottom) of collagen

Figure 5: HPLC to quantify drug

Figure 4: Drug release study using USP type II apparatus.

Release Media: PBS pH 6.8. Media volume- 500 mL

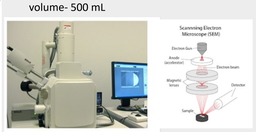


Figure 3: SEM for morphological analysis

RESULTS & DISCUSSIONS

Table 1. Nitrogen to HydroxyProline (N/HyP) ratio of collagen A, B and C

Implant	N/HyP ratio
A	1.18
B	1.19
C	1.18

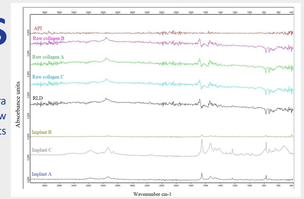


Figure 6. FTIR spectra of API, RLD, raw collagen and implants from sources A, B & C

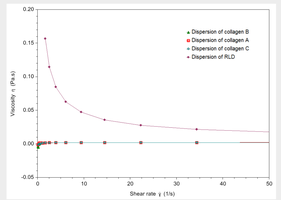


Figure 7. Rheology of resuspended RLD dispersions and drug collagen dispersions A, B and C

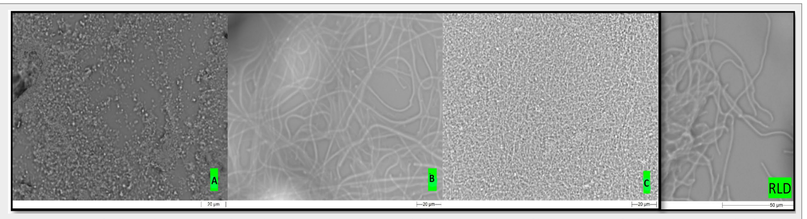


Figure 8. MDRS images of collagen dispersions A, B & C without drug and RLD dispersion: (a) loose clustered micro fibrillar structure of collagen dispersion A, (b, c) large interconnected tangled fibrillar structure of collagen dispersions B and C, (d) RLD. The fibrillar structure was not affected by changing mixing speed and mixing time

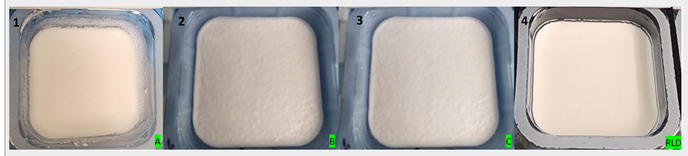
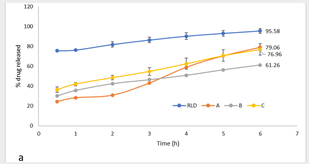


Figure 9. Photographs of RLD and lyophilized implants. 1: Implant from Source A showing rough surface and adhered to the container; 2 and 3: Implant from Source B and C showing smooth surfaces and were not sticking to the containers; 4: RLD implant



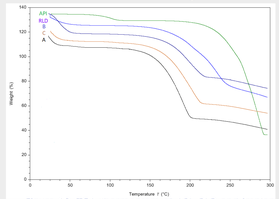


Figure 10. TGA thermogram of API, RLD and implants A, B and C

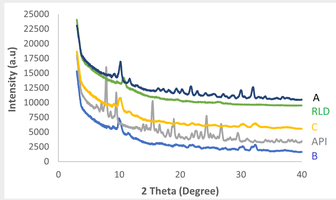


Figure 11. X-ray diffraction patterns of API, RLD and implants A, B and C

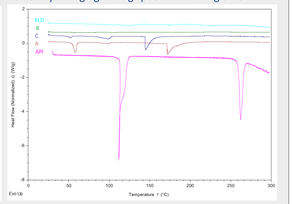


Figure 12. DSC thermogram of API, RLD and implants A, B and C

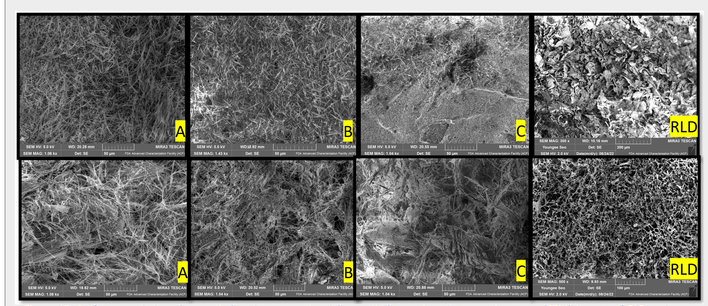


Figure 13. Surface morphology (upper tier) and cross sectional (lower tier) scanning electron microscopy (SEM) images of RLD and implants A, B and C

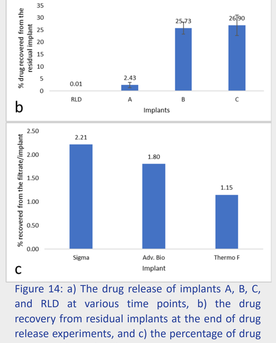


Figure 14: a) The drug release of implants A, B, C, and RLD at various time points, b) the drug recovery from residual implants at the end of drug release experiments, and c) the percentage of drug recovered from the filtrate

Characterization results of Raw Collagen

The results in Table 1 show that hydroxy proline ratio of raw collagen A, B and C were 1.18 , 1.19 and 1.18, respectively and no significant difference were observed ($p>0.05$).

The FTIR spectra in Figure 6 revealed that collagens from all sources exhibited similar peak patterns regardless of sources of collagen.

Characterization results of Collagen Dispersion

The MDRS images in Figure 8 revealed that the homogenization conditions (speed and time) did not impact the collagen fibrillar size in collagen dispersions without drug prepared using collagen A, B and C. However, the dispersions exhibited different fibrillar morphology. Image (a) show loose clustered micro fibrillar structure of collagen dispersion A, Images (b & c) show large interconnected tangled fibrillar structure of collagen B and C. Image of RLD dispersion show fibrillar structure comparable that of collagen dispersion B.

The results in Figure 7 show that all collagen dispersions A, B and C exhibit a low viscosity (exhibiting Newtonian flow). On the other hand, the RLD dispersion exhibited a non-Newtonian behavior with shear thinning effect.

Characterization results of Lyophilized Implants

Figure 9 show photographs of three implants—A, B, and C—manufactured from collagen A, B, and C. All implants exhibited complete formation and firmness. Notably, Implants B and C displayed smooth surfaces and were not sticking to the containers. On the other hand, Implant A exhibited a rough surface and adhered to the container. SEM images in Figure 13 show 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.

The moisture content were determined by TGA. The moisture content among the implants were 6.25% for A, 11.84% for B, and 6.97% for C, 11.25%) for RLD and 8.72% for drug (Figure 10). It is noteworthy that the moisture content of Implant B and RLD were similar. The Implant A and C contained significantly lower moisture levels.

The XRD diffraction patterns shown in Figure 11 revealed that the characteristic crystalline peaks of API were at 7.788°, 8.288°, 10.628°, and 14.268°, while these peaks were absent in RLD indicating amorphous nature of drug in RLD. The Implant A also exhibited similar crystalline peaks of drug. On the other hand, 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. The DSC results in Figure 12 indicate that Implant B and RLD contain drug in amorphous form, while the Implants A and C contain drug in crystalline or semicrystalline form.

Figure 6 displays FTIR spectra of the implants, demonstrating similar peak patterns among Implants A, B, and C, and RLD, with characteristic wavenumbers at 1500-1650 cm-1 (polypeptide backbone C-N stretching), 1700-1800 cm-1 (polypeptide backbone C-O stretching), 2900-3000 cm-1 (N-H in amide stretching) and 3200-3500 cm-1 (N-H in amide stretching) cm-1. Hence, no significant structural differences were observed.

The preliminary drug release results in Figure 14a 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%. 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 13b). About 1-2% of the drug per implant was recovered from the filter before lyophilization.

CONCLUSIONS

The structural properties of collagen from various sources appeared to be similar but the morphology and microstructure of the resulting implants were different which may explain the differences in drug release and in drug loss during filtration. Further studies to comprehensively examine the mechanism of drug 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

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