Characterization of animal soft tissue for development of a new *in vitro* model for drug release in intramuscular injections

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ABSTRACT

When studying the release of active pharmaceutical ingredients (API) in intramuscular injections of particulate drug delivery systems, mechanical and viscoelastic properties of the model biomaterial of soft tissue are often overlooked. In this study, viscoelastic properties of animal soft tissues were compared to those of various hydrogels in order to establish a development criterion for an improved *in vitro* model for measuring the API release in intramuscular injections.

INTRODUCTION

The release mechanism of an active pharmaceutical ingredient (API) is known to be highly dependent on the mechanical properties of the model biomaterial[1-3]. Nevertheless, matching the mechanical and viscoelastic properties of the model to the corresponding soft tissue is often overlooked when studying the release of API in intramuscular injections of particulate drug delivery systems. Noyes-Whitney equation describes the intrinsic release of a drug[3]:

$$\frac{dM}{dt} = -D \cdot A \cdot \frac{C_s - C}{h} \quad (1)$$

where dM/dt is the rate of dissolution, D is the diffusion coefficient, A the surface area, h is the thickness of the boundary layer and C_S and C are the saturation solubility and concentration at a time t, respectively.

Stokes-Einstein equation[3] describes the diffusion coefficient:

$$D = \frac{K_B \cdot T}{6 \cdot \pi \cdot r \cdot \eta} \quad (2)$$

where K_B is the Boltzmann constant, T is the absolute temperature, η the viscosity of the dissolution media and r the radius of the (spherical) drug particle. Eq. 1 and Eq. 2 combined show the reverse correlation of the intrinsic drug release rate and the viscosity of the material.

A muscle-like model structure containing the components of native tissue may be useful as a diffusion medium for *in vitro* investigation of transport phenomena. In this context, hydrogels can be used as muscle mimics for different applications, including tissue engineering[4, 5], as well as artificial tissue models for drug release and transport studies[6, 7]. Table 1 gives an overview of the shear modulus for various biological tissues and culture substrates.

Table 1. Shear modulus of tissues and culture substrates

Material	***G (Pa)***
Brain, nerves	10^2–10^3
Liver, fat, relaxed muscle, breast gland tissue	10^3–10^4
Dermis, connective tissue, contracted muscle	10^5–10^6
Epidermis, cartilage	10^7–10^8
Polyacrylamide gels	10^3–10^4
Bone, polystyrene	10^8–10^{10}

This study focuses on analyzing the rheological properties of animal muscle tissue that resembles the target tissue for intramuscular injections. Additionally, some hydrogels that have been used as tissue mimics, as well as new, modified hydrogels are investigated, and the viscoelastic properties (storage and loss modulus: *G'* and *G"*) of the animal tissue are compared to them. The objective is to establish an improved *in vitro* model for measuring the API release from particulate delivery systems administered by intramuscular injection.

MATERIALS AND METHODS

Bovine and porcine muscle tissue were collected a day before the measurements. The samples were cut into disks with a thickness of 3 mm and diameter of 40 mm, either perpendicular or parallel to the muscle fibers. The samples were stored at 4°C before the tests.

Scanning Electron Microscope (SEM) images of freeze-dried samples were taken using Hitachi TM3030 tabletop SEM (Spectral Solutions AB) after a 21 h freeze drying cycle.

Rheological measurements were conducted in small-amplitude oscillatory shear (SAOS) using a DHR-3 rheometer, (TA instruments) with a plate-plate geometry. Double-sided adhesive tape on the upper plate and a cross-hatched lower plate were used to prevent slip. Each sample was kept at 37°C between the plates for 2 min prior to measurement. As an exact and even thickness throughout a soft tissue is difficult to achieve without freezing the sample before cutting, the gap was adjusted for each sample by approximately 10 % compression from the position where the upper plate touches the sample surface. Frequency sweeps were conducted at constant strain of 0.1% and strain amplitude sweep at constant frequency of 1 Hz.

Rheological properties of the tissue samples were compared to those of various hydrogels:

1. Gellan gel: Gellan 0.5 % (w/v) and HPMC 0.5 % (w/v) in water, mixed and protonated in order to induce gelation. Thereafter the gels were neutralized by immersing in phosphate buffer.
2. Agarose gel: 0.5 % (w/v) agarose was suspended in phosphate buffer, heated up to the boiling point, cooled down for gelation, and measured at room temperature.
3. Cross-linked chitosan: Chitosan 1 % (w/w) was dissolved in a cold, dilute, aqueous hydrochloric acid (0.5 M) and stirred for 12 h at 4 °C. The pH of the solution was adjusted with the addition of fresh glycerol 2-phosphate disodium hydrate (GP, 9 % w/v) and stirred for 1 h at 4 °C[8].
4. Gelatine gel: Gelatine 14 % (w/w) was suspended in phosphate buffer at room temperature, the solution was heated to 50 °C which was maintained for 1 h. The rheological measurements were conducted during cooling.
5. Gelatine foam: Gelatine 14 % (w/w) solution was prepared as described above. The solution was cooled to 32 °C and whipped for 2 h, resulting in a thick foam (a patented method from Ferrosan).
6. Poloxamer gel: Poloxamer 407 17 % (w/w) was prepared by dissolving the required amount of P407 in cold water and stirring at 4 °C for 24 h[9].
7. Poloxamer+R-polox: The solution was prepared similarly to the Poloxamer, i.e.

P407 17 % (w/w) and 25R4 (reversed poloxamer, 1:1 molar ratio) were dissolved in cold water and the solution was stirred at 4 °C for a minimum of 24 h[9].

Agarose, chitosan, gelatin gel and foam, and both poloxamer samples were measured using the cone-plate geometry, 1° cone angle and 60 mm in diameter, whereas gellan gel was measured using the same setup as for the tissue samples.

RESULTS AND DISCUSSIONS

Analysis of the muscle tissue

SEM was used to analyse the structure of the muscle tissue. For this purpose the samples were freeze dried in a 21 h freeze drying cycle. The fiber structure and the difference between samples cut parallel and perpendicular to the fibers in the muscle tissue can be clearly seen by SEM (Figure 1).

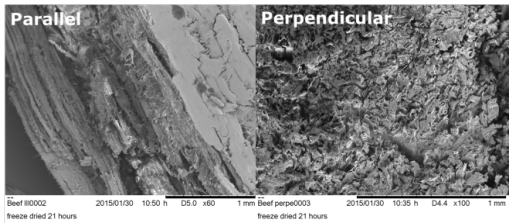

Figure 1. SEM images of the freeze dried bovine muscle tissue.

Strain amplitude sweeps indicated approximately the same linear viscoelastic range for all the samples (measured at ω= 1 Hz), *G'* showing the onset of nonlinear behaviour at around γ= 0.5 % (data not shown). The strain amplitude of 0.1 % was used for the consecutive SAOS measurements, where the storage moduli of the tissue samples ranged from 5.7 to 35.4 kPa at 1 Hz (Fig. 2). The absolute value of complex modulus $|G^*|$, calculated from *G'* and *G''*, ranged from 5.9 kPa to 36.5 kPa (1 Hz), corresponding well with the shear modulus range given for a relaxed muscle (Table 1). Additionally, a similar frequency dependency was observed for all the samples.

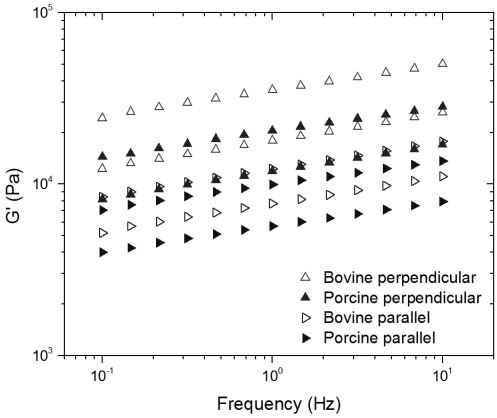

Figure 2. *G'* vs. frequency of the tissue samples: highest and lowest measured values (n=3-4).

The perpendicular cut samples showed higher moduli compared to the parallel cut, indicating stiffer structure in the direction cross-sectional to the muscle fiber. However, considering the large deviation between the repeated measurements, the significance of the differences can be debated. Figure 3 shows the median storage and loss moduli of each tissue sample. The bovine tissue samples showed in general somewhat higher moduli than the porcine tissue. Nevertheless, considering again the large deviation between the repeated measurements, typical for greatly inhomogeneous biological samples, the difference between the bovine and porcine tissue may be of less importance.

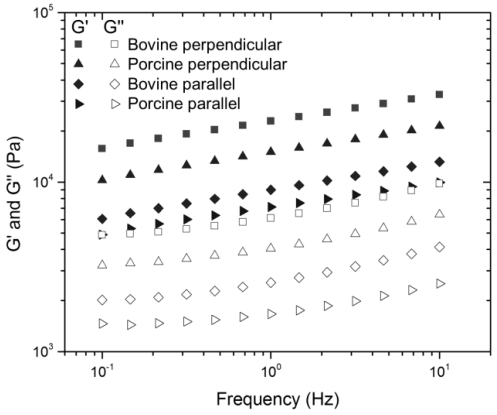

Figure 3. *G'* and *G"* vs. frequency: a representative (median value) of each tissue sample.

Comparison to hydrogels

Various hydrogels and gelatine foam were compared to the tissue samples. For the sake of simplicity, only the *G'* and *G"* of the perpendicular cut porcine tissue was used as a reference (Fig. 4).

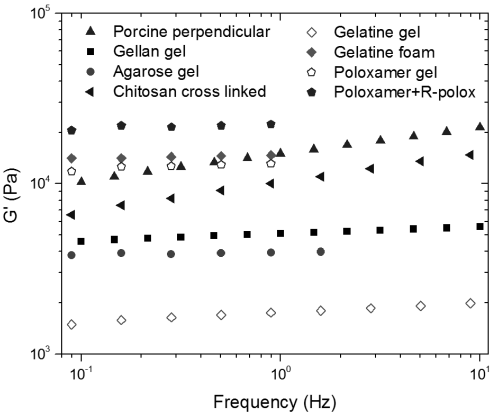

Figure 4. *G'* vs. frequency, different hydrogels compared to the perpendicular porcine tissue.

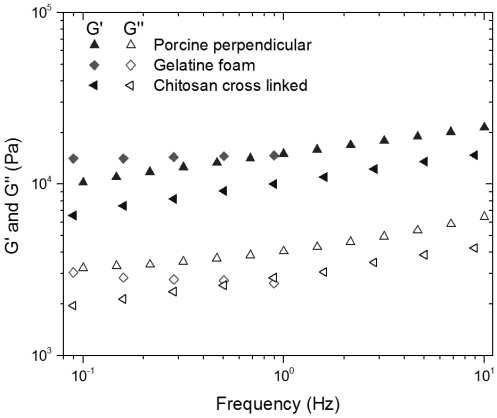

Figure 5. *G'* and *G"* vs. frequency: gelatine foam and the cross-linked chitosan gel compared to the perpendicular porcine tissue.

The gelatine, agarose, and gellan hydrogels all had too low value of *G'* and were thus regarded as unsuitable mimics for the muscle tissue. Both the poloxamer samples showed a sufficient value of the *G'* whereas the *G"* was very low and thus neither these samples were considered as good representatives for muscle tissue. Thus the gelatine foam and the cross-linked chitosan gel were studied further. The *G'* and *G"* of muscle tissue samples are closer to the moduli of gelatine foam than cross-linked chitosan gel (Fig. 5 and Table 2). However, the gelatine foam seems to be frequency independent, whereas the tissue samples as well as the cross-linked chitosan gel show frequency dependent behaviour. To quantify the difference, the power law exponent (n) was calculated for the selected samples by the equation using the complex viscosity (η^*):

$$\eta^* \propto \omega^{-n} \qquad (3)$$

where ω is the frequency. The power law exponent, and thus the frequency dependency of the chitosan sample correlates well with the power law exponent of the porcine tissue, whereas the gelatine foam has a power law exponent close to 1, indicating a frequency

independent, solid-like homogenous gel[10, 11], which is apparently not the case for the tissue sample.

Table 2. Measurement temperature, storage and loss moduli and power law exponent of the tissue sample (porcine perpendicular), gelatine foam and cross-linked chitosan gel.

Sample	**Porcine**	**Gelatine**	**Chitosan**
Temperature	37 °C	32 °C	37 °C
G' (0.5 Hz)	13.3 kPa	14.5 kPa	9.1 kPa
G" (0.5 Hz)	3.7 kPa	2.7 kPa	2.6 kPa
n	0.84	0.98	0.81

Tissue mimics should be constructed from a chemically similar material and possess physico-chemical properties that can be related to the structure of muscle tissue. Rheological characterization can give a good indication of the viscoelastic properties that are relevant for the tissue mimics for modelling of drug delivery. In this study the gelatine foam and the cross-linked chitosan gel showed characteristics similar to the animal tissue. However, both of these mimics have downsides: the gelatine foam has the chemical structure, i.e. building blocks of amino acids, similar to the muscle tissue, but it is opaque and thus not optimal for visual tracing of the drug. In addition, the porosity induced by the foaming may not provide same distribution routes for the injected media, as the cell structure in the tissue. The cross-linked chitosan gel, on the other hand, is transparent thus enabling visual tracing, but the structure is based on polysaccharides, being thus far from the chemical structure of the muscle. Last but not least, network structure of neither of the systems resembles the fiber structure of the muscle tissue. Nevertheless, the viscoelastic properties of these systems were similar to those of animal muscle tissue. Thus they possess potential for further development resulting in an optimized tissue mimic for the evaluation of the release of an API from particulate delivery systems administered by intramuscular injection.

CONCLUSIONS

In this study the rheological properties of animal muscle tissue samples were analysed in order to find out the viscoelastic properties required for muscle tissue mimics for studying intramuscular injections. The results were compared to rheological characteristics of several hydrogels and gelatine foam. The most promising tissue mimics were gelatine foam and the cross-linked chitosan hydrogel. However, the opaque and porous appearance of the gelatine foam, as well as the chemical composition of the cross-linked chitosan are not optimal for the modelling of intramuscular injections, thus further optimization is needed for improved *in vitro* models.

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