Freeze-dried chitosan formulations for mixing with platelet-rich plasma to form implants for tissue repair

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Main topic: Basic science
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Purpose: The purpose of this study was to develop freeze-dried chitosan formulations that can be solubilized in platelet-rich plasma (PRP) to form injectable implants for different tissue repair applications.

Materials & Methods: Several polymer formulations containing increasing chitosan M_n (number average molar mass) and concentrations, and excipient concentrations (as lyoprotectant), as well as calcium chloride (as clot activator) were freeze-dried. Leukocyte-rich PRP was collected from human donors (n=5) and used to reconstitute the freeze-dried formulations. The following performance characteristics were assessed: solubility (assessed visually), handling properties (runniness test), coagulation (thromboelastography), clot retraction (liquid expression) and clot homogeneity (histology). Freeze-dried formulations were also reconstituted in autologous PRP for dorsal subcutaneous injections into New Zealand White rabbits (n=11) to assess implant biodegradability and biocompatibility. Controls were recalcified PRP.

Results: Freeze-dried polymer formulations containing low and medium chitosan M_n and concentrations were rapidly and completely solubilised in PRP. Chitosan-PRP formulations were more viscous than PRP controls (Fig. 1a) and coagulated quickly (Fig. 1b) to form solid hybrid clots (Fig. 1c&d), which retracted much less than PRP controls (Fig. 1e). Chitosan dispersion in the hybrid clots was strongly dependent on the chitosan M_n, with medium M_n chitosan yielding homogenous clots (Fig. 1f to h). Chitosan M_n, chitosan concentration and excipient concentration modulated the performance of formulations (Fig. 2). Chitosan-PRP hybrid clots were internalized by host cells and resident until at least 2 weeks *in vivo* (Fig. 1i&j), while PRP controls were quickly degraded within days. No adverse reactions were observed post-implantation.

Conclusion: Freeze-dried chitosan formulations can be solubilised in PRP to form injectable self-gelling biodegradable and biocompatible implants for tissue repair applications. Unlike PRP alone, these hybrid implants are physically stable and significantly prolong bioactivity for tissue repair. Ongoing experiments are investigating their use in meniscus repair, cartilage repair and rotator cuff repair.

Keywords: Chitosan, platelet-rich plasma, injectable implants, tissue repair, biodegradability, biocompatibility

Figures:

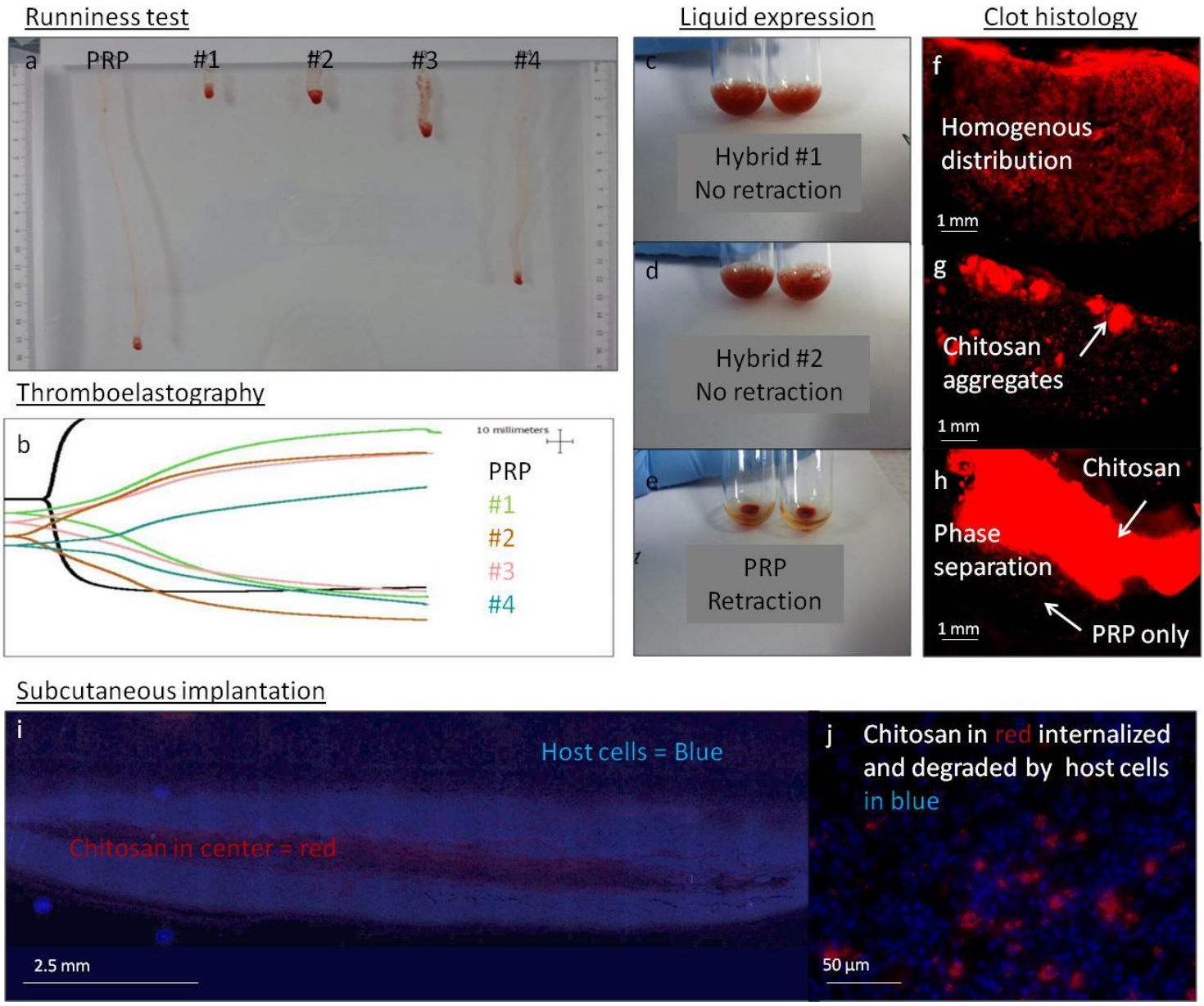

Figure 1. Example of a runniness test showing that different chitosan-PRP formulations (numbered 1 to 4) had paste-like properties and were more viscous than PRP alone (a). Example of a thromboelastograph test showing tracings for different chitosan-PRP formulations (in color, numbered 1 to 4) and for PRP alone (in black) (b). Chitosan-PRP hybrids remained voluminous after clotting (c&d) while PRP-only clots retracted significantly and exuded serum (e). Chitosan dispersion in the hybrid clots was homogenous when chitosan of medium M_n was used to prepare the freeze-dried formulations (f), but not when chitosan of high M_n (g) or of low M_n (h) were used. Chitosan-PRP implants were resident for at least 2 weeks *in vivo* and were degraded by host cells without any adverse reactions (i&j). Chitosan can be detected with red fluorescent microscopy in panels f to j since a rhodamine-chitosan tracer of similar M_n was added to each freeze-dried formulation for imaging purposes.

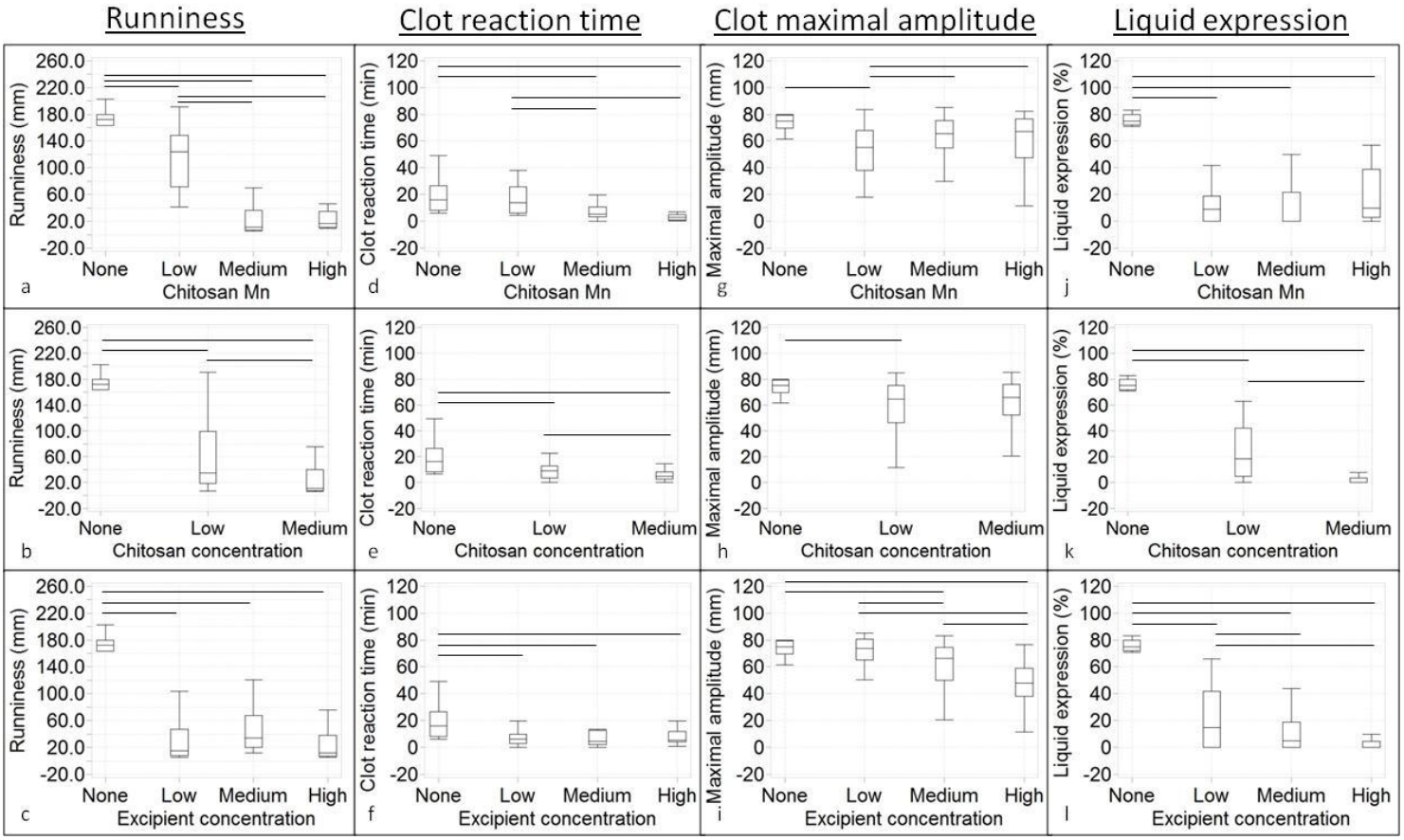

Figure 2. Increasing chitosan M_n and chitosan concentration improved paste-like properties of chitosan-PRP formulations (a&b). Increasing chitosan M_n and chitosan concentration decreased clot reaction time of chitosan-PRP formulations (d&e). Excipient concentration had no effect on runniness and clot reaction time (c&f). Hybrid clot maximal amplitude was similar to PRP alone for formulations containing chitosan of higher M_n at higher concentration (g&h). Increasing excipient concentration decreased clot maximal amplitude (i). All chitosan-PRP clots expressed less serum than PRP alone (j - l). Increasing chitosan and excipient concentration decreased liquid expression (k&l). The Mixed model task in SAS Enterprise Guide 5.1 and SAS 9.3 were used to compare the different groups with post-hoc analysis to look at pair-wise differences. Fixed effects were chitosan M_n, chitosan concentration and excipient concentration while donor was a random effect, as some donors were sampled more than once. $p < 0.05$ was considered significant. Data in the figures are presented as median (line); Box: 25^{th} and 75^{th} percentile; Whisker: Box to the most extreme point within 1.5 interquartile. Significant differences between pairs are indicated by the horizontal lines.