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Original Article

The Use of Thromboelastography to Measure the Influence Inclusion of a Local Anesthetic Agent has on the Mechanical and Kinetic Properties of Fibrin

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Abstract

Context: Delivery of slow-release local anesthesia has considerable potential for postoperative analgesia. Fibrin gel has shown huge potential for drug delivery, but has not been fully investigated for the delivery of local anesthetics nor has whether incorporation of anesthetic drugs into fibrin alters its mechanical properties. **Aims:** This study aimed to evaluate the effects of bupivacaine inclusion on the mechanical and kinetic properties of fibrin as measured by thromboelastography (TEG). **Materials and Methods:** Serial dilutions of fibrinogen with thrombin were tested with TEG to identify the optimal concentrations to give reproducible results. Following this, fibrinogen samples diluted with bupivacaine 0.5% in place of normal saline (also 1:20 dilution) were added to thrombin to assess what influence this had on clot strength and kinetics as measured by TEG values (with *R*, *K*, and α angle relating to clot kinetics and MA and *G* (or shear elastic modulus strength) relating to clot strength). **Results:** The mean values yielded for *R* were higher and lower for α angle, suggesting that the inclusion of bupivacaine produced a fibrin clot at a slower rate. The values for MA and G were both lower when bupivacaine was included, suggesting inclusion of the local anaesthetic also resulted in a fibrin clot of inferior strength. These results were not statistically significant. **Conclusion:** Although TEG failed to consistently measure these properties, the results suggest that inclusion of local anesthetic affects the clotting process of fibrin, potentially interfering with its ability to function as a sealant, adhesive, or hemostat.

Keywords: Fibrin delivery, local anesthesia, thromboelastography

Introduction

There is an identified need within pain management to optimize the delivery of local anesthetic agents.[1] Fibrin gel has shown huge potential for both cell delivery[2] and the local delivery of drugs including antibiotics[3] and chemotherapeutic agents.[4] The use of fibrin gel to deliver local anesthetics has not as of yet been fully investigated but has been suggested as a potential vector for slow release.[5] To evaluate this technique, it is important to assess the impact inclusion of a drug may have on the mechanical properties and efficacy of the fibrin gel, as inclusion of a local anesthetic agent should not render the fibrin ineffective and should allow for the sustained release of local anesthetic at a therapeutic concentration. It should ideally retain sufficient strength to resist shear and stress and still function as a hemostat, sealant, or adhesive as necessary depending on the clinical scenario. Inclusion of a local anesthetic agent to fibrin ideally should not result in any modification of the clotting process and change its overall properties. This study investigates the effect of bupivacaine on the mechanical and kinetic properties of fibrin as measured by thromboelastography (TEG). TEG has previously been validated as a method of providing reliable, reproducible information on clots generated by commercial fibrin.[6,7]

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Materials and Methods

Materials

A commercial fibrin glue with Food and Drug Administration approval as a tissue adhesive was used (ARTISS, Baxter,

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UK). It was supplied as a kit consisting of two prefilled syringes that contained a sealer protein solution (91 mg/ml fibrinogen and a 3000 IU/ml of a synthetic aprotinin) and thrombin solution (4 IU/ml diluted in 40 μmol/ml calcium chloride). Bupivacaine hydrochloride, 0.5% w/v solution for injection, was the local anesthetic agent used (Marcaine, AstraZeneca, Ireland), a long-acting agent typically used in wound infiltration catheters and nerve blocks.

Preparation of fibrin samples

Initially, multiple samples of fibrinogen with thrombin were tested with TEG to identify the optimal concentrations to give reproducible results. As reported,[6,7] TEG was unable to measure the strength of clots from undiluted fibrinogen and thrombin. Clot formation occurred too quickly and the strength was beyond the calibrated range. Therefore, the fibrinogen component of the fibrin was diluted with normal saline to obtain a concentration measurable by TEG, as had previously described.[6] Several serial dilutions were performed (1:10, 1:20, and 1:40). The 1:20 dilution component (18 μl of fibrinogen added to 342 μl of normal saline) was most consistently measurable by TEG and yielded results most comparable to the published results.[7] Therefore, 180 μl of the 1:20 fibrinogen dilutions was added to 180 μl of thrombin to produce 360 μl fibrin gels [Table 1].

The thrombin component was not diluted. These acted as the controls for the study. Following this, fibrinogen diluted with bupivacaine 0.5% in place of normal saline (also 1:20 dilution) was added to thrombin to assess what influence this had on clot strength and kinetics as measured by TEG.

Thromboelastography

The TEG device used was a TEG® 5000 Thrombelastograph® Hemostasis Analyzer system. This consisted of a temperature-controlled chamber with two separate workstations. It was calibrated to the standard provided by the manufacturer. A 360 μl sample is placed into a preloaded cup in each workstation. Although this technology is designed to evaluate the clotting process of whole blood, these parameters have been applied to fibrin. The following five parameters derived from TEG tracing (a thromboelastogram) can relate to fibrin clot formation.[6,7]

- *R* – mean time to initial clot formation and is measured in minutes (min)
- *K* – time from beginning of clot formation until it reached a predefined degree of elasticity (until the amplitude of TEG reaches 20 mm). This represents the dynamics of clot formations and is also measured in minutes (min)
- Alpha (α) angle – the angle between the line in the middle of TEG tracing and the line tangential to the developing "body" of the TEG tracing. It represents the acceleration of kinetic of fibrin buildup and cross-linking. Therefore, a greater angle indicates faster clot formation kinetics. It is measured in degrees (°)
- MA – this is the maximum amplitude of the tracing and represents the ultimate clot strength. A higher amplitude indicates a stronger clot. It is measured in millimeters (mm)
- *G* – this is a log-derivation of the mean amplitude. It is obtained from the following formula: 5000 MA/(100 − MA).[6] It is measured in dynes/second where dynes correspond to the centimeter-gram-second unit of force. Its value represents the ultimate clot strength often referred to as the shear elastic modulus strength (SEMS).

R, *K*, and α angle relate to clot kinetics and MA and *G* (or SEMS) relate to clot strength [Figure 1].

Data analysis

Data entry and analysis used Microsoft Excel software version 14.4.0. Mean values for parameters obtained by TEG were compared using a paired, two-tailed *t*-test with a significance level of 0.05 using Minitab software version 1.2.0, Minitab Express™.

Results

Of note, not all samples tested were measurable by TEG (7/12, i.e., 58.3% of fibrin samples, and 5/12, i.e., 41.7% of fibrin with bupivacaine). No specific differences could be identified between the samples measurable by TEG and those not. The *K* value (the time for clot to reach a predefined strength) was not measurable by TEG in any of the samples, as was described in Hickerson *et al*.'s study.[8] It is likely that TEG was unable to give values for *K* as the predefined strength applies to whole blood and is not easily achieved by fibrin. The remaining TEG values – *R*, α angle, MA, and *G* – for fibrin and fibrin with bupivacaine samples were compared [Table 2]. The mean values for *R* were higher and lower for α angle, suggesting that the inclusion of bupivacaine produced a fibrin clot at a slower rate. The values for MA and G were both lower when bupivacaine was included, suggesting inclusion of the local anaesthetic also resulted in a fibrin clot of inferior strength. These results were not statistically significant.

Table 1: Fibrin gel components

Solution	Volume (concentration)	Total amount
Fibrinogen	180 μl (1:20 dilution of 91 mg/ml)	0.82 mg
Thrombin	180 μl (4 IU/ml)	0.72 IU

Table 2: Mean (standard deviation) thromboelastography values for fibrin and fibrin with bupivacaine

TEG parameters	Fibrin	Fibrin with bupivacaine	*P*
R	1.5 (1.1)	2.06 (1.6)	0.61
K	NA	NA	NA
α angle	44.3 (4.3)	38.62 (6.4)	0.11
MA	18.96 (13.7)	4.64 (2.2)	0.07
G	1.34 (1.3)	0.24 (0.1)	0.21

SD: Standard deviation, MA: Maximum amplitude, NA: Not available

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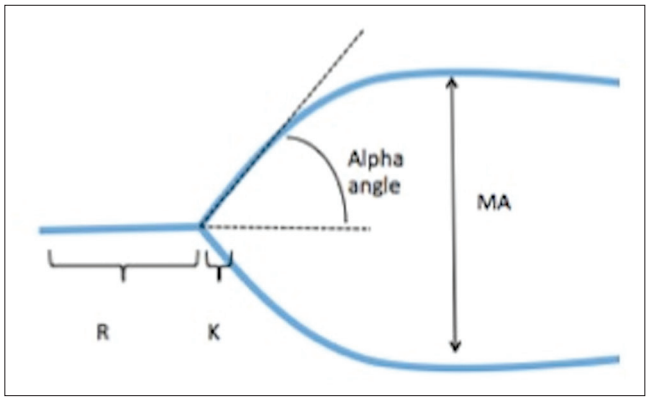

Figure 1: A thromboelastogram with *R*, *K*, α angle, and MA parameters illustrated

Discussion

The potential for fibrin gels to deliver local anesthetic agents has not been fully investigated, nor has whether incorporation of anesthetic drugs into fibrin may alter its mechanical properties. In the context of this study, TEG failed to consistently and reliably measure the mechanical and kinetic properties of fibrin. Preliminary testing suggested that TEG values could only be obtained for specific serial dilutions of fibrinogen. However, despite using a dilution shown to work elsewhere,[9] we struggled to persistently measure samples with TEG. Only 58.3% of fibrin samples and 41.7% of fibrin with bupivacaine samples tested at this concentration were measurable. The reasons for these inconsistencies were considered. In the first instance, it is possible that the unpredictable TEG values were a product of the TEG machinery. The reproducibility of the results from TEG technology has been questioned previously. In Chitlur *et al*.'s study, comparing data from 17 machines in nine international laboratories showed significant variation between laboratories and instruments, which the authors attributed to operator variability.[8] Despite these findings, it is still considered an effective measurement of fibrin-sealant properties due to its proven ability to compare serial dilutions of samples over time, suggesting that it is the relative values rather than absolute values yielded that are most useful. In addition to this, it is an entirely closed system that constantly monitors clot kinetics throughout the clot formation process. Second, there may have been difficulties with the fibrin clot itself. Previous studies in this field were limited. From their findings and our preliminary testing, we aimed to identify an optimal dilution of both fibrinogen and thrombin for consistent clot formation; however, this was not achievable. It is likely in this study that there was too much dilution with a critically small concentration of fibrinogen or thrombin that would not permit measurable polymerization. Extensive testing with multiple serial dilutions of fibrinogen and thrombin may allow for more consistent and reproducible results. Finally, the inclusion of the bupivacaine could have been the source of the unpredictable results. The mean values for *R* were higher and lower for α angle in fibrin with bupivacaine samples; therefore, its inclusion produced fibrin clots at a slower rate. The values for MA and G were both lower when bupivacaine was included, suggesting inclusion of the local anaesthetic also resulted in a fibrin clot of inferior strength. Although not statistically significant, the data demonstrated a weakening of thromboplastic properties, suggesting that the included bupivacaine is acting as more than simply a diluent. The reason for this impact on either the initial polymerization of the fibrin monomer or the subsequent cross-linking to form a fibrin clot could potentially be due to variations in pH. The deterioration of the clotting function of fibrinogen has been previously described with pH reductions.[9,10] The inclusion of bupivacaine, a weakly acidic agent, could potentially modulate the overall pH and therefore contribute to changes in clot kinetics.

Conclusion

Although TEG failed to consistently and reliably measure the mechanical and kinetic properties of fibrin, results yielded from this pilot study suggest that the inclusion of a bupivacaine adversely affects the fibrin clotting. This may have implications in terms of fibrin's efficacy as a sealant, adhesive, or hemostat. As part of a complete evaluation of fibrin as a drug delivery agent for local anesthesia, further investigations with larger studies are recommended.

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Conflicts of interest

There are no conflicts of interest.

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