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Ultrasonic machining of biomass using biodegradable slurry

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ABSTRACT

Thrombi, *e.g.* blood clots, in circulatory system pose acute health risk, globally. This research investigated roles of biodegradable starch slurry in advancing biomass machining efficiency. Hard clots (fibrin-rich) prepared from rabbit blood were exposed *in vitro* concomitantly to ultrasound (1 MHz) and starch slurry. Starch slurry particles (diameter ~250 nm) yielded a 200% increase in material removal (sonothrombolysis) efficiency. Mechanistic participation of starch, a non-Newtonian material, at the interface of biomass-ultrasonic radiation is discussed. Overall in subtractive biomanufacturing, the role of biodegradable slurry is critical for enhancing material removal efficiency.

1. Introduction

At present, cardiovascular diseases (CVDs) are the leading cause of human deaths in the world [1,2]. In year 2008, 17.3 million people died of CVDs [1,2], the number is expected to reach 23.3 million by 2030 [1,3]. When observed mechanically, CVDs are caused mainly due to anomalies suffered by blood flow in circulatory channels, effecting change in blood pressure. The major obstacle in the blood flow paths of the circulatory system is created by the *in vivo* development of blood clots (thrombi representing biomass). Blood clots can develop because of various undesirable reasons, including work-induced immobility [4]. For example, blood clots can develop in the veins of the legs (Deep Vein Thrombosis, DVT) [1,5,6], and transported to the lungs *via* blood flow (Pulmonary Embolism, PE) [1]. Traumatic conditions of DVT and DVT derived PE, attack travellers across the world daily.

The stochastic, uncontrolled parametric nature of non-diagnosed thrombosis (blood clotting process) is a state of health risk, which demands similar levels of urgency in both therapeutic (cure) and preventive medical biomanufacturing sectors. The most common and FDA approved drug to combat the risk posed by clots is the intravenous (IV) administration of recombinant tPA (tissue plasminogen activator, alteplase; Activase from Genentech, Inc., U.S. license # 1048; 2002) [7]. tPA is an enzyme which converts plasminogen (inactive precursor) to plasmin (active enzyme), and when administered in larger doses as in traumatic cases (proportionate to clot mass and distribution) further debilitates the situation by causing derived traumatic effects such as extravasation (leakage) of blood into surrounding tissues (*e.g.*, haemorrhage). When observed unequivocally, tPA aided thrombolysis significantly acts only on the fibrin network of the clot, and not directly on to interlocked blood corpuscles (*e.g.*, erythrocytes, red blood cells; leukocytes, white blood cells; platelets). Ideally the blood clot an *in vivo* heterogeneous volumetric blockage (*i.e.*, natural biocomposite) must be cleared of all the contents at a nearly uniform material removal rate (MRR), which can be facilitated by simultaneous removal of clot bulk (fibrin mesh and interlocked cells), contrary to just unravelling the interlocked cells *via* fibrinolysis. To achieve a nearly uniform MRR the chemically intensive process of thrombolysis has to be modified by the introduction of an in-process mechanically active material removing agent.

Ultrasonic energy is used as a therapeutic agent for treating thrombosis. Ultrasonic energy in conjunction with microbubbles forms the key drivers of sonothrombolysis process, where use of tPA is optional [8]. The above-discussed concerns of tPA induced after effects could be addressed by experimenting with new medium capable of enhancing efficiency of mechanical and chemical material removal. The medium needs to be able to efficiently transfer ultrasonic energy to cells and polymerized protein network together forming blood clot, at the same time act as a mechanical "abrasive/ablative" medium and must be biocompatible. This research proposes and tests sonothrombolysis process efficacy by using starch slurry of sub-micron size particles acting as a key medium in a set-up similar to ultrasonic machining (USM) process commonly used for machining hard and brittle engineering materials. Starch is a known non-Newtonian [9] and biodegradable material [10].

Based on the current knowledge-base of ultrasonic machining and its application in diverse fields and need for enhancing process

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efficacy of sonothrombolysis, a study has been conducted to explore potential of starch as a machining medium, to increase the biomass material removal rate (*i.e.*, clot loss %), and for potentially limiting the use of drugs (*i.e.*, tPA) for arresting symptomatic issues such as hemorrhage. A non-contact tool (radial horn) was used to energize the material removing media for investigating its effect on increasing sonothrombolytic efficacy. Feasibility of sub-micron scale particles forming the starch slurry acting like abrasive particles used in ultrasonic machining process to enhance the material removal rate or blood clot loss is studied.

Second section briefly presents basic principles, parameters and applications of USM and discusses its feasibility in applying to blood clot removal. Experiments are detailed in Section 3 with results and discussion in Section 4. Conclusions of this study are presented in Section 5.

2. State-of-the-art

In ultrasonic machining process abrasives (such as boron nitride, silicon carbide and diamond) contained in slurry are accelerated at a high velocity against the workpiece by a reshaped tool vibrating at low amplitude (about 0.076 mm) and at high frequency of 20–40 kHz). USM equipment comprises of a tapered horn (sonotrode, booster) linking the transducer and the pre-shaped tool [11]. Small size abrasive grits are used in micro-USM (250 nm–3 μm) [12], and large size grits are used in macro-USM (5–180 μm) [13]. Sonotrode assisted ultrasonic energy (waves) impacts and vibrates the slurry media in the tool-workpiece gap (approximately equal to the abrasive grit mean size) [14], producing a precise mirror image of tool shape on workpiece surface. The vibrating grits hammer on to the workpiece promoting the development and propagation of microfractures and material dislocations, eventually flakes of brittle workpiece separate from crack boundaries and disintegrated material bulk [14].

In the medical field, tPA based thrombolysis is a biochemical therapeutic process, removing blood clots *via* disintegration of the fibrin network [15]. Clinically adopted, an improvement over standalone drug based thrombolysis is the chemomechanical clot removal process, sonothrombolysis. In sonothrombolysis ultrasonic waves (1–3 MHz) are used in presence of tPA, where inclusion of microbubbles (3 μm) in media further enhances the clot loss % [8]. Microbubbles pulsate (compress and distend) in continuum with ultrasonic stress wave (compression and rarefaction). Pulsating microbubbles create eddies (microstreaming, fluid movement) across the work surface aiding in the development of shear stresses [16]. The sonothrombolytic mechanisms governing material removal are not fully understood. Red blood cells are observed to disintegrate under the influence of ultrasonic waves, forming microspheres [16]. The ultrasonic tools used in sonothrombolysis are of two types- contact mode and non-contact mode. Commercially available catheters (*e.g.*, EKOS EndoWave) are the ultrasonic tools directly coming in contact with the blood clot, transferring energy for clot disintegration [17]. Non-contact tooling comprises of the commonly available ultrasound equipment based on Doppler shift phenomena, used for measuring blood flow *i.e.*, Transcranial Doppler (TCD) and Transcranial Color Coded Sonography (TCCS) operated with ultrasonic horns for transmitting energy through the skin barrier [18]. High frequency waves in 1–3 MHz range usually face attenuation (intensity loss) while passing through bones as in skull (cranium), with added adversity of heat generation. Unlike in manufacturing industry lower frequency use (in KHz) raises concerns of hemorrhage.

3. Experiments

Following experimental section discusses preparation of starch slurry and testing set up applied for sonothrombolysis. Understanding the morphology, chemistry and distribution of starch particles in medium is important for conducting controlled sonothrombolysis machining experiments.

3.1. Synthesis and characterization of starch slurry

Unlike in USM where abrasive particles of known size are mixed with water to form abrasive slurry, in this study potato starch powder (S2004; Sigma–Aldrich) was used as a material of choice. The processed starch particles were dispersed in deionized water (10 wt% solids). Subsequent to stirring, the solution thus formed was sonicated for 30 min using bench-top sonicator/cleaner (Branson 1510 R-DTH, 40 kHz). After sonication the solution was filtered using PES (polyethersulfone) bottle top filter with 0.45 μm pore size (Nalgene). The filtration step was monitored *via* vacuum pump–pressure control/gauge assembly (UN811KV.45P; KNF LAB) connected to the bottle top filter. During filtration the vacuum was varied from ~2 psi to 8 psi yielding nearly complete filtrate, *i.e.*, starch slurry. The slurry was characterized using cryo-transmission electron microscopy, *i.e.*, cryo-TEM (TF20, FEI), and particle size analyzer (Partica, Horiba Scientific) for registering the morphology and size distribution of starch submicron–to- nano size particles. Fig. 1 shows cryo-TEM image of starch slurry sample, confirming uniform particle size distribution. The nanoparticulate population comprised of particles lying approximately between 115 and 584 nm (output from Partica). 97.78% of population formed sub-390 nm sector, with 90.70% of particles ranging between 150 and 339 nm. Cryo-TEM confirms shape/morphology of representative individual starch particles, rounded as well as faceted, where particles along with water medium are fixated in a frozen sample state for high resolution TEM analysis.

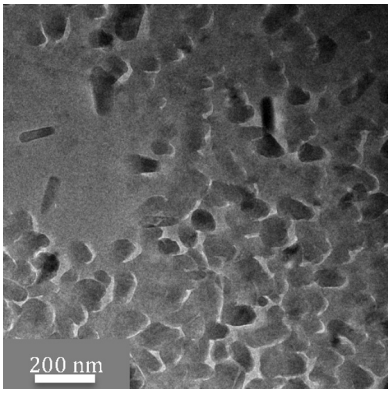

Fig. 1. Cryo-TEM micrograph of starch particulates dispersion.

3.2. Synthesis and characterization of sonothrombolytic media

As a part of preparing machining medium for blood clot, starch slurry was further diluted with phosphate buffer saline (iron-supplemented Bovine calf serum with inherent tPA), to form the thrombolytic media in a biomanufacturing experiment. In this second step, it is equally important to perform particle size analysis again, especially in a diluted and active bovine calf serum medium. In case, starch slurry was used for media preparation after a period of 10 days (rest) following the slurry synthesis, an extra sonication step was added to form a translucent dispersion, to provide check over agglomeration. Absorption spectroscopy was used to characterize the sonicated starch slurry.

3.3. In vitro *sonothrombolysis using starch slurry*

Mettler Toledo radial horns (710, 740 models) were used as the ultrasonic wave emanating source. Rabbit model blood clots (Fig. 2) were placed inside a mylar straw (5.2 mm ID). Distance between horn and tube was kept constant at 6.5 cm, *i.e.*, energy source to workpiece gap. Syringe pump was used to dispense the thrombolytic contents into the tube. Horn and tube assembly were maintained at human body temperature, housed inside a water bath. Sonothrombolysis process used pulsed ultrasonic waves, with 1 MHz ultrasonic wave frequency (cycles per second, where