Forensic Science International 257 (2015) 242–251

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Design and evaluation of a bioreactor with application to forensic burial environments

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ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 8 June 2015
Accepted 22 August 2015
Available online 7 September 2015

Keywords:
Artificial aging
Bioreactor
Bone
Decomposition
Postmortem interval
Soil analysis

ABSTRACT

Existing forensic taphonomic methods lack specificity in estimating the postmortem interval (PMI) in the period following active decomposition. New methods, such as the use of citrate concentration in bone, are currently being considered; however, determining the applicability of these methods in differing environmental contexts is challenging. This research aims to design a forensic bioreactor that can account for environmental factors known to impact decomposition, specifically temperature, moisture, physical damage from animals, burial depth, soil pH, and organic matter content. These forensically relevant environmental variables were characterized in a soil science context. The resulting metrics were soil temperature regime, soil moisture regime, slope, texture, soil horizon, cation exchange capacity, soil pH, and organic matter content. Bioreactor chambers were constructed using sterilized thin-walled polystyrene boxes housed in calibrated temperature units. Gravesoil was represented using mineral soil (Ultisols), and organic soil proxy for Histosols, horticulture mix. Gravesoil depth was determined using mineral soil horizons A and Bt2 to simulate surface scatter and shallow grave burial respectively. A total of fourteen different environmental conditions were created and controlled successfully over a 90-day experiment. These results demonstrate successful implementation and control of forensic bioreactor simulating precise environments in a single research location, rather than site-specific testing occurring in different geographic regions. Bone sections were grossly assessed for weathering characteristics, which revealed notable differences related to exposure to different temperature regimes and soil types. Over the short 90-day duration of this experiment, changes in weathering characteristics were more evident across the different temperature regimes rather than the soil types. Using this methodology, bioreactor systems can be created to replicate many different clandestine burial contexts, which will allow for the more rapid understanding of environmental effects on skeletal remains.

1. Introduction

Accurate estimation of the postmortem interval (PMI) provides context to forensic investigations. The PMI defines the time elapsed between death and discovery of human remains [3]. Methods for estimating PMI of recovered remains with soft tissue present, which can be observed for up to 1285 average degree days (ADD) or the equivalent of a PMI of approximately six months or less, have received more attention than methods applicable after complete skeletonization has occurred [4]. However, precise methods for PMI estimation from skeletal remains continue to elude researchers [1–4]. Change in citrate concentration is a novel method being developed in forensic research that utilizes the biochemistry of bone specimens to estimate PMI [1,2]. Currently there are no methods that provide a robust methodology for evaluating the impact of environmental variables on changes in biochemical properties of bone in a burial context.

Skeletal remains are exposed to diverse microenvironments that result in varied bone weathering patterns and decomposition processes. Different areas on a single bone can exhibit different stages of bone weathering and decomposition depending on whether or not a given bone area has ground contact (soil exposure) [5,6]. Skeletal remains recovered from a burial context with full soil contact provide particular challenges for estimating

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http://dx.doi.org/10.1016/j.forsciint.2015.08.014
0379-0738/

PMI; however the unique properties of the soil at a burial site also provide the opportunity to examine environmental variables for modeling the decomposition process in different geographic locations. Benninger et al. [7] suggested that soil-based methods are potentially useful for estimating PMI over extended timeframes. Methods for systematically examining the effects of soil and related environmental variables on decomposition are lacking and are the focus of the current study.

In order to develop methods for estimating PMI that are not site specific to a given geographic region and based on case study observations, a careful experimental design approach is needed. Variables known to impact the rate of decomposition must be isolated and evaluated independently. Environmental modeling databases provide information about the most important variables related to decomposition, specifically temperature, moisture, and soil properties for nearly every location in the United States and for most worldwide locations. In this manner, the impact of a particular set of variables and environmental characteristics can be evaluated to better understand changes in the biochemical properties of bone over time [2]. By gaining an understanding of these factors, methods proposed for estimating PMI from buried skeletal remains can be broadly applied across regions with different environmental conditions.

Decomposition is categorized into five phases based on the physicochemical and bacterial environments: autolysis, putrefaction, liquefaction, dissolution, and skeletonization [8]. Soft tissues break down due to microbial decomposition and natural cell death occurs during the first four phases, while bone diagenesis occurs due to mechanical breaking, decalcification, and dissolution in acidic soils or water during dissolution and skeletonization phases. Taphonomy uses the study of ecology to identify environmental factors that can lead to body decomposition after death [9]. The primary taphonomic factors include: water movement, temperature regime, organic matter content, fluoride and carbonate concentration, duration of internment in soil, soil pH, cryoturbation, microbial activity, release of fat, and mineralogy [10,11]. Using a mechanistic approach to understand the distinct variables in this complex system, these taphonomic factors can be systematically controlled in a forensic bioreactor to empirically determine their impact on bone biochemistry.

Bioreactors are technical systems that enable reproducible and controlled operating conditions and support biologically, chemically, or physically active processes. Bioreactors are generally defined as devices in which biological and/or biochemical processes develop under closely monitored and controlled environmental and operating conditions (e.g. pH, temperature, pressure, oxygen content, nutrient supply, waste removal) [12–14]. In the current study, the term "forensic bioreactor" describes a technical system used to monitor, control and manipulate environmental factors that impact decomposition. Carter et al. [14] successfully implemented a forensic temperature incubation system over 28 days for the assessment of rat cadaver decomposition with respect to temperature (15–29 °C) and microbial activity. In a shorter term study, Abdel-Maksoud [15] implemented an archeological temperature incubation system over 13 h for the assessment of bone specimens with respect to temperature (200–300 °C). Both of these studies attempted to identify an environmental mechanism for the changes seen in cadaver specimens [14,15]. Carter et al. [14] found that temperature acted as an environmental mechanism and increased microbial activity, which is known to impact decomposition. Abdel-Maksoud [15] found that temperature as an environmental mechanism impacted coloration and crystallinity in such a way that artificially aged bone samples could be matched to archeological bone samples. Based on the results of these studies, the simulation of other environmental mechanisms and evaluation of their impact on decomposition would aid in understanding how cadaveric samples progress in burial conditions over time. Through the design of a forensic bioreactor system capable of controlling existing parameters known to impact decomposition, many different burial scenarios could be considered side by side.

Design parameters for existing bioreactor systems are well described, thus a system can be designed to incorporate discrete variables relevant to forensic taphonomy. Common design parameters include: size of the apparatus, temperature, sterility, oxygenation, ability to apply sensors, use over time, transparency of the bioreactor, easy placement of the sample, and easy replacement of the medium [17]. Given the chemical properties that soil imparts to the system, soil becomes the medium for a forensic bioreactor [11]. Similarly, as microbial activity is a hallmark of decomposition processes, the bioreactor can be used for its intended purpose – the cultivation of microbial populations [12]. In evaluation of the apparatus, chamber type, sample type and sample size must be considered.

The overall purpose of the current study is to design and develop a forensic bioreactor as a method for controlling key environmental variables affecting bone decomposition, specifically temperature and soil, in order to systematically evaluate their impact on bone biochemistry over time. A synergistic collaboration between relevant disciplines is pursued, including bioengineering to define the biosystems control of the bioreactor, forensic anthropology to define factors impacting bone decomposition, and soil science to define the soil types and related environmental variables (Fig. 1). The specific objectives are: (1) to identify the environmental factors most important for modeling burial settings in an outdoor context that are useful for forensic anthropology; (2) to discretize environmental factors of an outdoor forensic burial setting using soil science data; and (3) to design a forensic bioreactor that can be easily monitored to mimic an outdoor burial setting.

2. Materials and methods

2.1. Defining a baseline of environmental factors through forensic taphonomy

Human remains in clandestine burials are interred in gravesoils. These soils define the environments that forensic specimens are exposed to during decomposition and lead to the acceleration or

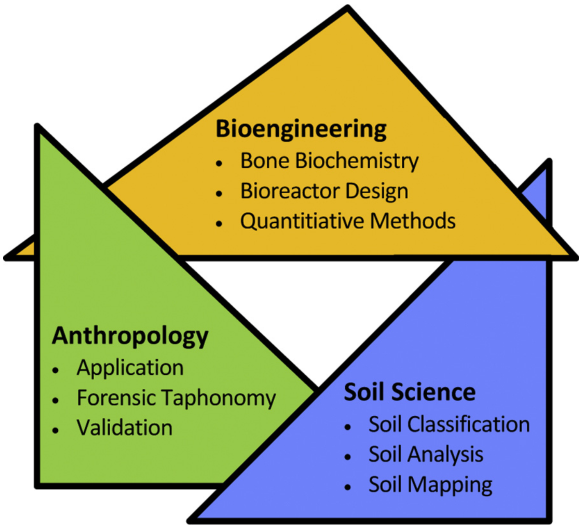

Fig. 1. Identifying the synergy between collaborating fields: anthropology, bioengineering, and soil science.