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Original Article

A new tissue slice technique for evaluation of cardiac tissue enzymes and metabolites

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Abstract

This study describes the fabrication of a simple yet efficient tissue slicer, consisting of a five-razorblade holder mounted inside a syringe. The device is designed to produce uniformly sized tissue slices in a single, precise motion. By aligning the razor blades at equal distances, researchers can easily slice through biological tissues, ensuring consistent thickness across all samples. Detailed instructions on the assembly and operation of the device are provided, highlighting its ability to streamline the tissue preparation process. This method offers a cost-effective and reliable solution for generating uniform tissue sections, making it particularly valuable for metabolic and biochemical studies where consistency is crucial for accurate experimental results.

Keywords: slice technique, apparatus, evaluation studies

1. Introduction

The practice of using tissue slice preparations to investigate physiological and biochemical phenomena has a long history, dating back to the 1940s (Haynes, 1965; Stadie & Riggs, 1944). This method has proven to be a valuable tool in scientific research, enabling the study of various tissue-specific functions under controlled experimental conditions. Specifically, cardiac tissue slices have practically been prepared using microtomes, which are precision instruments designed to cut extremely thin slices of tissue for microscopic examination or biochemical assays. Despite the availability of advanced microtome techniques, the use of a simple slicing technique for tissue preparation continues to be favoured in many laboratories due to its ability to produce slices of uniform thickness. This ensures consistency in experimental results, as evenly sliced tissue allows for more reliable data collection. Furthermore, compared to the more specialized and complex Stadie-Riggs microtome tissue slicer (Stadie & Riggs, 1944), which was specifically developed for preparing tissue slices, the simpler instrument setup is much more cost-effective and practical for everyday use. Its ease of operation and lower maintenance requirements make it a more accessible tool, particularly for researchers who may not have access to advanced equipment, yet still need to obtain high-quality tissue samples for their studies. Thus, while innovations in tissue slicing technologies continue to emerge, the conventional setup remains a widely used and valuable instrument in scientific research, providing an efficient, affordable, and reliable means of preparing tissue slices for the examination of physiological and biochemical processes.

The preparation of cardiac tissue slices requires distinct techniques that differ from those consistently employed for preparing coronal slices of other tissues, such as brain slices. Each method has its own specific challenges and considerations, tailored to the unique structural and functional properties of cardiac tissue. In general, the process of preparing cardiac tissue slices using specialized techniques is more time-consuming compared to the free-hand slicing method used by Stadie-Riggs microtome (Stadie & Riggs, 1944). Free-hand slicing, while faster, may result in less precision when it comes to the uniformity of the tissue thickness. However, when precision is prioritized, particularly for research applications that demand high consistency in tissue slices, using more controlled techniques, such as those involving a microtome, tends to offer better results.

The slower, more methodical approach typically ensures that the slices are of more uniform thickness, which is

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crucial for experiments where consistency is essential for accuracy and reproducibility. Studies on cardiac electrophysiology, metabolism, or drug response often require slices with uniform dimensions to ensure that experimental results are not biased by variations in slice thickness, which could impact the diffusion of nutrients, oxygen, or experimental compounds.

Despite the additional time required, both techniques, whether the slower, more controlled method or the faster free-hand approach are versatile enough to be used for preparing slices from the entire heart, as well as from specific regions of interest. This flexibility allows researchers to focus on particular areas of the heart, such as the ventricles or atria, depending on the focus of their study. The specific investigations might require targeted slices from regions where certain pathological changes are localized, or where the response to stimuli is expected to vary from other parts of the heart. Both methods, when applied properly, can yield useful slices for a wide range of experimental purposes, from functional studies to biochemical analyses, enhancing our understanding of cardiac physiology and disease.

2. Materials and Methods

The free-hand slicing method, used by the Stadie-Riggs microtome (Stadie & Riggs, 1944), is used frequently. A razor blade within a commercially available cartridge (Figure 1) is pressed into a cross-section of the barrel of a 20ml or appropriate injection syringe. It is being fixed firmly with adhesive (Figures 2 and 3). The razor blades are fixed at an incline to the barrel for easy and smooth slicing. After the setup is complete, the chick heart can be excised, and cleaned by removing fat tissue and blood clots, and the cleaned tissue can be carefully loaded inside the barrel (Figure 4).

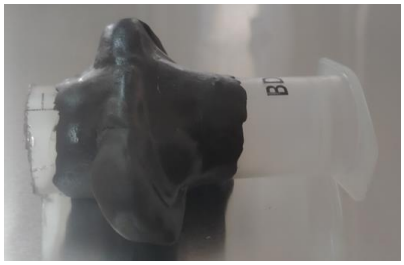
As the plunger is being pressed gently against the tissue-loaded barrel (Figure 4), the sharp razor blades will slice the tissue and produce thin slices of a specific thickness (Figure 5). Figure 4 illustrates the concept based on the standard procedure that can be used for cardiac tissues of chick and small rodents like mice and rats. The organ can be trimmed when larger cardiac or any other tissues from rabbits or guinea pigs are to be used. Different sizes of barrels and razors can be assembled and fastened with adhesive. With modest practice, satisfactory tissue slices can be prepared quickly using this procedure.

2.1 *In vitro* experiments on tissue slices

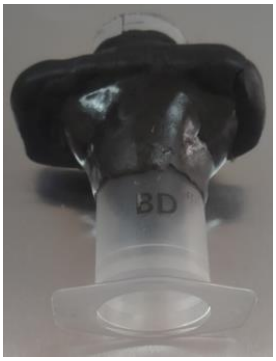
The study of metabolic processes for *in vitro* surviving tissue slices are always tied to tissue series experiments and statistical evaluation of the results. Incubation of tissue slices and specific treatment with agonists or antagonists are well-known and proven methods for the evaluation of biochemical properties of the tissue slices from organisms (Haynes, 1965). As a demonstration of the technique, a series of tissue slices obtained using this method is analysed for a few biochemical metabolites and enzymes over a period of time. Thus, the excised chick heart tissue slices are kept in 3 ml of ice-cold Krebs-Ringer-bicarbonate (KRB) buffer, pH 7.5 (Figure 6).

Figure 1. Razor blade within cartridge

Figure 2. Syringe with razor blade setup (inner view). Commercial multi razor blade mounted on the cut portion of the barrel with blades assembly kept inclined inwardly.



(a)



(b)

Figure 3. Razor blade mounted on the barrel space with blades facing inwardly. Adhesive was used to fix this setup firmly. Allowed to set overnight, (a) side view, (b) top view

Figure 4. The overall completed apparatus setup with tissue loaded barrel with plunger. Freshly excised chicken heart inserted into the barrel from the rear end.

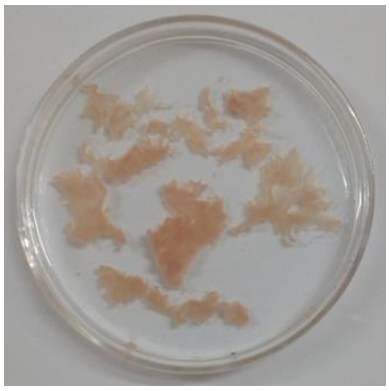


Figure 5. Heart tissue slices made using the assembled apparatus. Here, long slices of even thickness (0.5mm) of chick heart are kept in chilled Krebs-Ringer-bicarbonate (KRB) buffer solution.

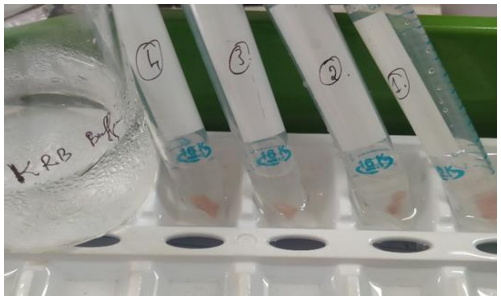


Figure 6. After performing slicing, the slices were separated immediately and kept in centrifuge tubes for individual washing with chilled Krebs-Ringer-bicarbonate (KRB) buffer solution.

2.2 Extraction of tissue slices

At the end of the incubation, the vessel was chilled, emptied into a conical centrifuge tube, and rinsed with 2 ml of cold KRB buffer (Pifree) and centrifuged at 1,200 *x g* for 2 min. The slices were washed once more with 5 ml of the same buffer. Then 0.8 ml of 10% trichloroacetic acid (TCA) was added to the slices and stirred well with a glass rod. After centrifugation, 0.3 ml of the TCA supernatant solution was used for estimating the glucose, pyruvate, lactate and lactate dehydrogenase (LDH) activities in the tissue (Wu, 1964).

Enzyme kinetics (forward and backward reaction) studies were done by Singh *et al.* (2012) method. Perturbation of Glucose assayed using Glucose oxidase method (Arkray AUTOSPAN Liquid Gold Glucose Kit) (Kaplan, 2005; Trinder, 1969; Roberts, Mcmillin, Burtis & Bruns, 2008a; Young, 1997; Kaplan, 1983), pyruvate by DNPH method (Moraes et al., 2016), lactate dehydrogenase by optimized DGKC kinetic assay method (Arkray AUTOSPAN Liquid Gold LDH Kit) (Pesce, 1984; Howell, 1979; Roberts et al., 2008b; Kaplan 1983; Buhl, 1978), and lactate by colorimetric method (Ahmadi, Lee, Oh, Park & Kwak, 2020) were evaluated by incubating the slices in a final volume of 2 ml of KRB buffer at 37°C.

2.3 Application and results

The preparation of cardiac tissue slices with precise thickness, such as 0.2 mm, poses significant challenges requiring technical expertise and specialized equipment. Achieving such thin, uniform slices is particularly difficult without the proper apparatus and considerable practice. On the other hand, slightly thicker slices, around 0.5 mm, are much easier to produce and more resilient during the subsequent experimental procedures, such as incubation. Thicker slices are less prone to fragmentation or structural disruption, which can occur during handling or incubation, making them more suitable for maintaining tissue integrity in prolonged studies.

Measuring the dimensions and thickness of the slices in this context is not always straightforward, and in practice, this measurement is often neglected due to the complexity of the setup. However, one common method to estimate slice dimensions involves floating the tissue slices over a buffer solution as shown in Figure 4. This allows researchers to trim the slices so that the areas can be measured more easily. From this point, the volume of the tissue slices can be calculated based on their wet weight, with an assumed specific gravity. With both the volume and area known, the thickness of the slices can be accurately determined.

The significance of this preparation technique goes beyond cardiac tissue and can be applied to a variety of organs. The tissue slice incubation studies provide dynamic insights into metabolic processes, which are crucial for understanding the fundamental metabolic characteristics and functions of the tissues under investigation. This information is particularly valuable in the study of disease pathophysiology, as it enables researchers to analyze how metabolic functions in tissues are altered under pathological conditions.

However, achieving uniform thickness in tissue slices across different studies remains a challenge due to the multitude of preparation techniques available, each of which depends heavily on the skill and experience of the person performing the slicing. The method described in this study (Figure 4) offers a key advantage by reducing the time required for tissue preparation and minimizing the variability introduced by the operator's skill. This method involves using

commercial razor blades housed in stainless-steel cartridges, which are manufactured with precise spacing and thinness, ensuring that the blades are perfectly aligned in a single plane. This design allows for a single cutting motion to produce two tissue slices, with a thickness variation of no more than 10% between them. The uniformity of this approach helps ensure consistent tissue preparation, reducing errors introduced by uneven cutting.

By applying smooth pressure on the plunger and maintaining a steady hand during the cutting process, researchers can obtain multiple tissue slices efficiently. The method enhances the reproducibility of slice preparation, which is critical for accurate and reliable experimental results. The plotted data (Figures 7-10) provide a clear demonstration of the metabolic activity observed in these tissue slices, particularly with respect to glucose, pyruvate, lactate, and lactate dehydrogenase (LDH) activities. As tissue amounts increase, the activity levels of these metabolic markers also rise, showing a pronounced tissue-dependent enzymatic response. Over time, between 5 and 15 minutes, glucose and pyruvate activities exhibit significant increases, especially in larger tissue samples, suggesting that more tissue mass leads to heightened enzymatic processes. Similarly, lactate and LDH activities follow a parallel trend, reflecting enhanced metabolic function as tissue concentration and incubation time increase. These findings underscore the tissue's metabolic dynamics and the cellular processes that intensify with greater tissue volume.

The consistency of these measurements is reflected in the low standard deviations observed in the data, which indicate that the results are reliable and reproducible across different experimental conditions. This consistency is crucial for validating the experimental model and drawing meaningful conclusions about tissue metabolism in various physiological and pathological contexts.

3. Discussion

According to Fuhrman and Field (Fuhrman & Field, 1945), the optimal thickness for rat liver slices used in metabolic experiments within the Warburg apparatus ranges between 0.48 and 0.62 mm. This specific thickness range is considered ideal for ensuring adequate diffusion of gases, nutrients, and metabolic substrates during incubation, which is crucial for obtaining reliable metabolic data. By using five separate sheets of razor blades, each with a thickness of approximately 0.3 mm, it is possible to generate tissue slices with a thickness of around 0.5 mm. These slices can span lengths of several millimeters, making them suitable for various experimental setups.

One of the significant advantages of this approach is the cost-effectiveness, especially when compared to more complex and expensive tissue preparations, such as those used for heart matrices. Heart slices, being easier to obtain and prepare, offer a more affordable alternative for metabolic studies, while also being easily disposable after a single use. This reduces the need for complex sterilization processes or re-use, streamlining the experimental workflow. Moreover, the preparation method is straightforward and can be conducted using simple tools, as described in this study, making it accessible even for laboratories with limited resources.

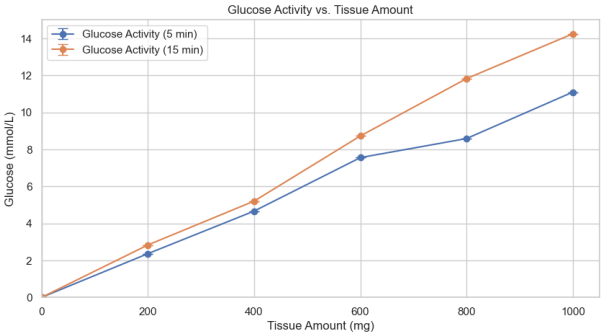


Figure 7. Graphical representation of Glucose activity in cardiac tissue slices of 200 – 1000mg

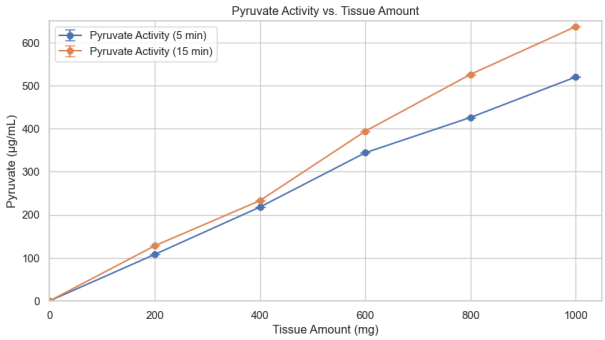


Figure 8. Graphical representation of Pyruvate activity in cardiac tissue slices of 200 – 1000mg

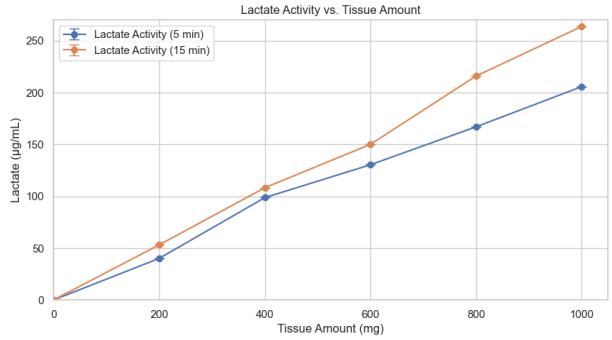


Figure 9. Graphical representation of Lactate activity in cardiac tissue slices of 200 -1000mg

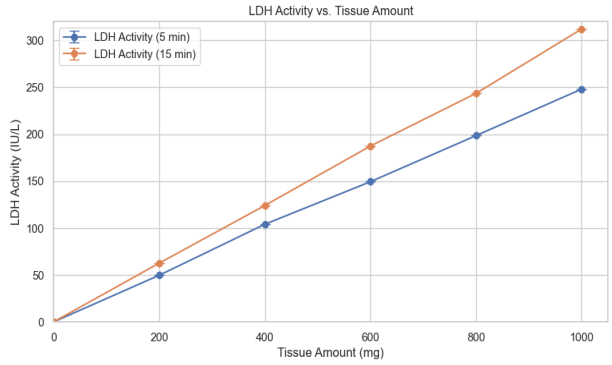


Figure 10. Graphical representation of Lactate Dehydrogenase (LDH) activity in cardiac tissue slices of 200 -1000mg

The slicing technique employed in this study follows a methodical approach that ensures uniformity and precision, factors that are crucial for obtaining consistent results in metabolic experiments. In our kinetic evaluation, the results obtained (as shown in Figure 11) were consistent with the findings reported by Singh *et al*. (2012), demonstrating that similar enzymatic activities can be reliably observed using the tissue slice method. This comparison highlights the validity of tissue slice techniques for assessing metabolic functions across different studies and experimental conditions.

The enzymatic activity of lactate dehydrogenase (LDH) is particularly relevant in this context, as it plays a key role in cellular metabolism by catalyzing the conversion of lactate to pyruvate and vice versa. The results indicate that LDH activity is sensitive to the concentrations of both lactate and pyruvate in the incubation medium. However, the relationship between LDH activity and lactate concentration is more complex and non-linear, suggesting that lactate influences LDH activity in a dynamic manner that may involve multiple factors, such as substrate saturation or allosteric regulation.

In contrast, pyruvate consistently reduces LDH activity as its concentration increases, indicating a more predictable and linear relationship. This suggests that higher levels of pyruvate inhibit LDH activity, likely due to competitive inhibition or feedback regulation within the metabolic pathways involved. The observed enzymatic behavior is crucial for understanding the metabolic dynamics especially in heart tissue, as LDH is a central enzyme in the regulation of glycolysis and the lactate-pyruvate equilibrium, which are essential for maintaining cellular energy balance.

Overall, the findings from this study align with previous research, such as that of Singh *et al.* (2012), reinforcing the reliability of tissue slice preparation methods for studying enzymatic activities in metabolic experiments. The tissue slice technique, when combined with precise kinetic measurements, offers valuable insights into the metabolic functions of heart tissue and other organs, providing a cost-effective and efficient method for conducting biochemical research.

4. Conclusions

In summary, this study presents a straightforward yet advanced tissue slicing device that is easy to assemble and highly effective for use in various biological experiments. The simplicity of this gadget does not compromise its precision or functionality, making it an ideal tool for researchers looking to prepare tissue slices with minimal technical complexity. This innovation in tissue slicing technology represents a significant step forward in experimental methodologies, enabling researchers to produce uniform slices efficiently without relying on complex machinery or advanced technical skills.

Historically, the work of Solomon and Schanker (1963) demonstrated the active uptake of organic bases by liver slices, marking an important discovery in the study of tissue transport mechanisms. Their research highlighted how liver tissue slices could be used to investigate the active transport of certain organic compounds, laying the foundation for future studies in this area. Despite these advances, earlier attempts to demonstrate the uptake of specific chemicals and organic acids by tissue slices had met with limited success. The challenge in effectively studying the uptake of these substances stemmed from the inherent complexity of their transport mechanisms within tissue slices.

However, this limitation should not be viewed as an insurmountable obstacle. Instead, it opens up new avenues for research, suggesting that with further refinement of tissue slicing techniques and experimental conditions, it is possible to gain a deeper understanding of tissue transport dynamics. The current study, with its simplified tissue slice preparation method, provides a critical tool for overcoming previous challenges. It facilitates more straightforward investigations into the transport properties of sliced tissues, enabling researchers to examine the potential of these tissues to uptake and process a wide range of compounds, including organic acids.

Moreover, this innovative approach to tissue slice preparation has the potential to bridge significant gaps in the field of metabolomics. The ability to study tissue slices in a controlled and reproducible manner opens up new possibilities for exploring how tissues metabolize various substances, how different compounds are transported into and out of cells, and how cellular processes are regulated at the metabolic level. This technique allows for more detailed studies of metabolic functions across different tissues, shedding light on the biochemical pathways that underlie both normal physiological processes and disease states.

The broader implications of this method extend beyond simple tissue slice preparation. Researchers must

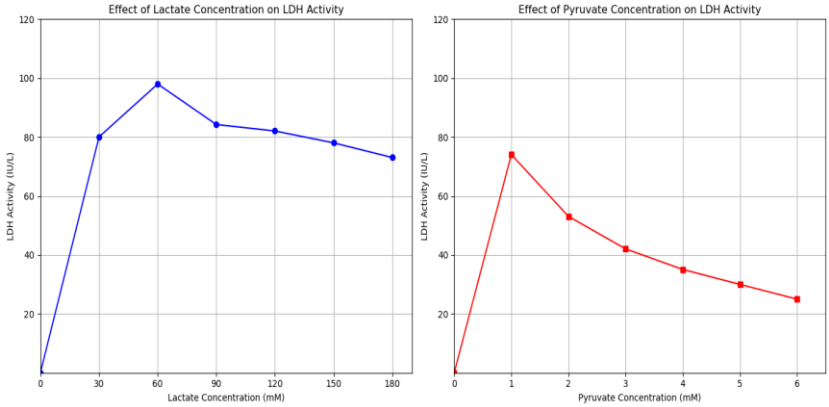


Figure 11. Effects of Lactate (30mM – 180Mm) and Pyruvate (1mM – 6 Mm) concentration on LDH activity in cardiac tissue slice

recognize the numerous possibilities that this technique offers for advancing our understanding of metabolomics and tissue transport mechanisms. By providing a reliable and accessible way to prepare tissue slices, this method paves the way for future discoveries in the field, particularly in areas where traditional tissue preparation techniques have fallen short. This could lead to significant breakthroughs in our understanding of metabolic regulation, cellular transport, and the pathophysiology of various diseases, ultimately contributing to the development of new therapeutic strategies and interventions.

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