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Microtubule destabilization with colchicine increases the work output of myocardial slices

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Abstract

Cardiac microtubules have recently been implicated in mechanical dysfunction during heart failure. However, systemic intolerance and non-cardiac effects of microtubule-depolymerizing compounds have made it challenging to determine the effect of microtubules on myocardial performance. Herein, we leverage recent advancements in living myocardial slices to develop a stable working preparation that recapitulates the complexity of diastole by including early and late phases of diastolic filling. To determine the effect of cardiac microtubule depolymerization on diastolic performance, myocardial slices were perfused with oxygenated media to maintain constant isometric twitch forces for more than 90 min. Force-length work loops were collected before and after 90 min of treatment with either DMSO (vehicle) or colchicine (microtubule depolymerizer). A trapezoidal stretch was added prior to the beginning of ventricular systole to mimic late-stage-diastolic filling driven by atrial systole. Force-length work loops were obtained at fixed preload and afterload, and tissue velocity was obtained during diastole as an analog to trans-mitral Doppler. In isometric twitches, microtubule destabilization accelerated force development, relaxation kinetics, and decreased end diastolic stiffness. In work loops, microtubule destabilization increased stroke length, myocardial output, accelerated isometric contraction and relaxation, and increased the amplitude of early filling. Taken together, these results indicate

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Ethics statement

The study was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Vermont.

CRediT authorship contribution statement

Emmaleigh N. Hancock: Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Bradley M. Palmer:** Writing – review & editing, Supervision, Software, Methodology, Data curation, Conceptualization. **Matthew A. Caporizzo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bradley M Palmer reports financial support was provided by IonOptix. Bradley M Palmer reports a relationship with IonOptix that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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that the microtubule destabilizer colchicine can improve diastolic performance by accelerating isovolumic relaxation and early filling leading to increase in myocardial work output.

Keywords

Cardiac microtubules; Colchicine; Diastolic dysfunction; Diastology; Myocardial slices

1. Introduction

Diastolic dysfunction (DDx) is estimated to affect roughly 12.7 % of the general population and is a precursor to diastolic heart failure which accounts for 1.3 % of people [1]. DDx arises from a combination of impaired relaxation and ventricular stiffening which has the early presentation of exercise intolerance [2]. Limited progress has been made in understanding the molecular underpinnings of DDx, largely due to challenges characterizing diastolic function in patients [3]. In addition, there are limited experimental techniques to assess diastolic performance of working myocardial samples under physiological conditions. Thus, there is a critical need for techniques to bridge the experimental assessment of myocardial mechanics with diastolic physiology.

Diastole is a complex process in which the contracted heart tissue relaxes and relengthens to commence refilling of the ventricular chambers with blood [4]. The amount of blood ejected during systole (stroke volume) is nominally equal to the amount of blood that enters the left ventricle during diastole, making diastolic performance and contractile performance equally important for determining cardiac output [5,6]. Diastole occurs in three separate phases: isovolumic relaxation (IVR), early filling (E), and late filling driven by atrial contraction (A) [7]. DDx is characterized by a prolonged IVR and an elevated end-diastolic pressure-volume relationship (EDPVR) that reduces late filling [8]. Reductions in E arise from impaired relaxation, and reductions in A arise from myocardial stiffening [9]. Clinically, the E/A ratio, which refers to the peak velocity ratio of early to late filling for blood entering the left ventricle measured via trans-mitral doppler, is used to “grade” diastolic function [10]. Echocardiography is an accurate non-invasive way to assess diastolic performance. However, true characterization of diastolic physiology, i.e., relaxation and stiffness, requires simultaneous measurements of ventricular pressure and chamber dimensions relating them as a cardiac pressure-volume (PV) loop. PV loops are the gold standard for the assessment of cardiac performance but are seldom conducted in clinical settings due to their invasive nature [11]. To assess tissue performance ex-vivo, a one dimensional force-length analog to the PV loop has been developed for myocytes and myocardial samples [12,13]. Recent advancements in FL loops precisely mimic the systemic vascular afterload providing a more physiological read out of systolic performance [14]. However, the advancement of FL loops to mimic diastolic physiology is still needed to assess diastolic performance ex-vivo.

An increase in the density of the microtubule network occurs in most forms of heart failure, in response to pressure overload, and in hypertrophic cardiomyopathy (HCM) [15-20]. Microtubules increase the stiffness of failing myocardium and provide a viscous impediment to contraction and relaxation [21-23]. The microtubule destabilizing drug colchicine is

derived from the *Colchicum autumnale* plant and has been used as anti-inflammatory agent since the ancient Egyptians [24]. It is commonly known for its treatment of gout, pericarditis and other inflammatory conditions and has garnered attention for reducing cardiovascular events [25]. Colchicine improves mechanical performance in animal models of heart failure and in human cardiomyocytes [15-18]. However, isolating the benefit of colchicine on diastolic performance from other systemic effects has been challenging. In patients, for example, colchicine's cardiovascular benefit is likely to stem from anti-inflammatory effects since higher than tolerable doses are required to destabilize cardiac microtubules. Thus, understanding the effect of microtubule destabilizing compounds such as colchicine on cardiac performance in isolation is of significant clinical importance for the development of microtubule-based therapies used in the treatment of heart failure [26].

Herein we leverage recent advancements in living myocardial slices to develop a technique to mimic diastolic physiology in working myocardial samples [27]. Using a force feedback system to generate myocardial work loops, we superimpose a trapezoidal stretch at the end of diastole that mimics atrial systole and allows us to assess the end-diastolic force-length relationship (EDFLR). In this way, our work loops contain information relating to all three phases of diastole: IVR, early filling and late filling. We found that a modified oxygenated DMEM/F-12 was sufficient to maintain isometric twitch force and their kinetics stable for extended durations of time. We used this system to investigate the effect of 90 min of colchicine treatment on diastolic performance and myocardial work. We found colchicine reduced diastolic tension and modestly accelerated twitch kinetics. In the working preparation, colchicine improved cardiac work, enhanced early diastolic filling, and accelerated isovolumic contraction and relaxation. Overall, these results indicate that microtubule destabilization with colchicine can exert a direct effect on the diastolic performance of working non-failing myocardium sufficient to improve stroke work.

2. Methods

2.1. Slice preparation

20-week-old female Wistar Kyoto rats (Charles River) were anesthetized with isoflurane and injected with Heparin (160 U/kg). Hearts were excised and placed in cold (4 °C) oxygenated (100 % O_2) dissection buffer containing 2,3-butanedione monoxime (BDM) (in mM: 140 NaCl; 5.4 KCl; 10 HEPES; 1.8 $CaCl_2$; 0.6 $MgCl_2$; 10 Glucose, 30 BDM) [28,29]. The atria and right ventricle were removed, isolating the left ventricle, which was opened by cutting down the septum to the apex. The left ventricular free wall was then mounted epicardial side down with Histoacryl glue (Tissue Seal, Ann Arbor, MI) onto a magnetic metal base and placed into the vibratome slicing chamber (Vibrating Microtome 7000SMZ-2, Camden Instruments, Loughborough, Leicestershire, UK) filled with oxygenated dissection buffer. Prior to each heart slicing, the stainless-steel blade was replaced, and Z-axis deflection was recalibrated to less than 1 μm. Tissue was sliced with settings at: 80 Hz vibrational frequency, 2.00 mm amplitude, 300 μm thickness, and 0.07 mm/s advance speed [27]. Slices from the mid-myocardium were then cut into 7 mm × 5 mm sections with myofibrils aligned lengthwise maintaining sections of identical dimensions (c.f. Supplementary Fig. 1). Previous studies have demonstrated that slices prepared by this method exhibit over 90 %

viability [27]. The sections were mounted onto custom laser cut plastic supports, cassettes, to ensure that tissues maintained their resting length, and resting sarcomere length, to start the experiments.

2.2. Work loop experiment

Myocardial slices were hooked to the force transducer and length controller of the Tissue Slice System (IonOptix, Westwood, MA) in recirculating room temperature (22 °C) oxygenated (100 % O_2) KHB (in mM, 140 NaCl, 4.5 KCl, 10 HEPES, 1.8 $CaCl_2$, 0.6 $MgCl_2$, 10 Glucose) and modified DMEM/F-12 media (powder DMEM/F12 (Cytiva, HyClone Laboratories, South Logan, UT SH30004.02), HEPES buffered and supplemented with $CaCl_2$ to 1.8 mM; to pH 7.4 with NaOH) by attaching the cassette to the system (Fig. 1). The edges of the cassettes were trimmed, and the slice was stimulated at 1 Hz for at least 15 min to equilibrate contractile function after which the isometric twitch forces reached steady state. Length and force measurements were captured with a length controller and force transducer via the IonWizard Software (IonWizard 7.7, IonOptix). Isometric twitches were collected and used to determine the preload and afterload settings in the work loops.

Work loops were configured with a preload set at resting force and afterload at 66 % of the developed peak isometric twitch force. A trapezoidal stretch to define the late phase of diastolic filling (200 ms ramp, 100 ms hold, and 100 ms return, 600 ms rest) was superimposed into the diastolic force clamp such that the 200 ms ramp occurs at the end of diastolic filling, i.e., electrical stimulus, to recapitulate stretching of the ventricle in late diastole by atrial systole. Thus, in this protocol we maintained fixed parameters of atrial contraction duration and relative amplitude. Parameters associated with late-filling speed and length will still change with myocardial stiffness, EDFLR. In addition, the timing of early filling will depend on tissue relaxation kinetics and thus spacing between E and A and E/A are free to change. The hold, return, and reset of the late-diastolic stretch occur in the background of the systolic force feedback and do not contribute to length changes. The amplitude of the trapezoidal stretch was set to 20 % of the preload aiming for the late-filling phase to account for about 30 % of the stretch in diastole at t0. The feedback gains (Kp) were deliberately set in IonWizard to respond with some lag during force development and relaxation so that the ejection and filling phases exhibited more physiological curvature rather than being sharply squared off. This corresponded to Kp of 200 (unitless) in IonWizard. Prior to DMSO or 10 μM colchcine treatment, t0 work loops were collected and then again after 90 min of treatment with tissues maintained at resting length under 1 Hz stimulus for the duration of the treatment.

2.3. Data management and analysis

For each set of isometric twitches and work loops force, length, and cardiac cycle phase values were taken from IonWizard software and saved as a txt file. Five sequential transients were averaged using a custom R script and exported as a CSV file into Origin software (OriginLab, Northampton, MA). Data points indicate individual tissue slices from $N = 5$ animals, with $n = 7$ DMSO and $n = 8$ colchicine 10 μm slices. Traces display mean line ± standard error and box plots display mean line, standard error box, and standard deviation whiskers. Statistical analysis was conducted between slices using paired *t*-tests between t0

and t90 within treatment groups and a two-sample t-test performed across treatment groups on the net effect of DMSO or colchicine, i.e. the change between t0 and t90 for DMSO was compared to the change between t0 and t90 for colchicine. *P*-values less than 0.05* are considered statistically significant. Origin rendered graphs and layouts were exported as SVG files to Adobe Illustrator (2022) for panel formatting.

3. Results

Force-feedback of tissue slices generate work loops of force vs. length that are analogous to the pressure vs. volume work loops obtained for clinical assessment of cardiac mechanics. To date feedback systems have a fixed preload and afterload that cannot accurately recapitulate the early and late phases of diastolic filling. We have addressed this by programming a mechanical stretch at the end of diastole to mimic late diastolic filling driven by atrial contraction (Fig. 1). This provides a readout of tissue stiffness analogous to the end-diastolic pressure volume relationship and by taking the derivative of length vs. time during diastole it provides peaks of early and late filling velocity that are analogous to those obtained from trans-mitral doppler echocardiography used to clinically grade diastolic function (Fig. 1C).

Traditional muscle mechanics experiments have largely been performed in simple phosphate buffered solutions containing glucose and chloride salts. While these solutions have been seminal to muscle mechanics experiments [29], we found that Krebs-Henseleit buffer (KHB) promoted strong initial force generation, but isometric twitch force was not stable over the 90+ minutes of continuous stimulation needed to test colchicine intervention. We found a gradual decay in isometric force (7.58 mN ± 1.52 to 3.00 ± 0.69 mN $p = 0.051$) and prolonged relaxation kinetics (0.22 ± 0.02 s to 0.32 ± 0.04 s $p = 0.015$*) after 90 min in KHB (Fig. 2D). However, in experiments performed using the modified DMEM/F-12 media isometric twitch force was remarkably stable with no significant changes in isometric twitch force or contractile kinetics (Fig. 2B-D). The long-term stability of the tissue in DMEM/F12 is critical to be able to accurately distinguish changes associated with colchicine treatment from tissue run-down during the protocol. Nevertheless, in all experiments, net effect within groups and between DMSO and colchicine treated samples were compared.

To determine if microtubule destabilization by colchicine (10 μm) treatment had any impact on myocardial force generation, isometric twitches were collected immediately preceding work loops at t0 (before treatment) and t90 (90 min after treatment). DMSO (1:1000) treatment for 90 min (Fig. 3A) had no impact on isometric twitch with peak height, time to peak, relaxation time, and diastolic tension remaining unchanged between t0 and t90 (Fig. 3). Colchicine treatment from t0 to t90 did not significantly change the peak isometric twitch force but did decrease passive diastolic tension (0.00 ± 0.01 mN to −0.89 ± 0.91 mN, $p = 0.027$*) (Fig. 3C). Colchicine treatment also accelerated contractile force development and relaxation rates, reducing time to peak force (0.18 ± 0.024 s to 0.15 ± 0.02 s, $p = 0.029$*) (Fig. 3C) and early (10 %), middle (50 %), and late (75 %) relaxation times (Fig. 3D).

Next, we wanted to determine how microtubule destabilization with colchicine might impact work output of the myocardium. Work loops were configured as described above with

preload, afterload, and late filling kept the same between t0 and t90. Tissue force was plotted against length from five averaged traces to generate a work loop for each tissue before and after treatment (Fig. 4A). Overall, the 90 min incubation with vehicle led to a leftward shift in the work loop without significant changes in stroke length, work and end diastolic elastance. Microtubule depolymerization with colchicine treatment shifted the work loop rightward towards longer lengths and increased tissue stroke length (0.05 ± 0.03 mm to 0.07 ± 0.02 mm, $p = 0.012$) (Fig. 4D) and work (1.30 ± 0.89 mN.mm to 1.91 ± 1.18 mN.mm, $p = 0.035$*) (Fig. 4E).

To identify the force-dependent and length-dependent effects of microtubule destabilization, force and length were plotted against time for a contractile cycle (Fig. 4A, B). While peak force was not changed by 90 min of colchicine treatment (Fig. 4F), colchicine accelerated contractile kinetics in the working slices reducing the time to peak force development (0.09 ± 0.02 s to 0.079 ± 0.02 s, $p = 0.030$) (Fig. 4G) consistent with the isometric twitch result (Fig. 3). In the length vs. time trace which clearly illustrates the shift towards longer diastolic length by colchicine, a reduced time spent and minimum length, and increased overall shortening (stroke length) was observed (Fig. 4C). End-diastolic elastance, approximated as the end-diastolic force/stroke length, was reduced (17.58 ± 10.47 mN to 0.51 ± 19.91 mN, $p = 0.041$*) (Fig. 4H), while the slope of the late diastolic filling phase (EDFLR or stiffness) was unchanged with either vehicle or colchicine treatment (Fig. 4I).

To better visualize how force generation and relaxation are impacted by colchicine in the working myocardium, the rate of force development (dF/dt) was derived from the force trace (Fig. 5). DMSO treatment for 90 min had no significant impact on the rate or amplitude of force development or relaxation. Colchicine, however, increased the rate of force production, maximum yank (dF/dt) (99.88 ± 47.78 mN/s to 137.78 ± 57.48 mN/s, $p = 0.031$) and the rate of relaxation, minimum yank, (−33.92 ± 15.87 mN/s to −55.79 ± 19.77 mN/s, $p = 0.003$*) (Fig. 5C). In addition, colchicine led to an earlier development of force and relaxation with maximum yank occurring faster and minimum yank trending towards occurring at earlier times (Fig. 5C-D).

Clinical assessment of diastolic function uses trans-mitral doppler echocardiography to measure the velocity of blood flow into the left ventricle during the early and late filling phases of diastole. We can mimic this in the working slice preparation by taking the time derivative of tissue length and plotting velocity over the working cycle (Figs. 1, 6A, B) Changes in early (E) and late (A) filling are used to provide a clinical grade of DDx with the ratio of peak E to peak A, the E deceleration time, and the duration of late filling commonly reported. While colchicine treatment showed a clear shift towards earlier and greater peak early filling, the late filling parameters of E/A and E deceleration time were unaffected by colchicine (Fig. 6C). A more in-depth breakdown of tissue velocity which includes the velocity-time integral (VTI), time to peak velocity, peak velocity, and duration for systole, early filling, and late filling (Fig. 7). Systolic VTI is negative as it represents shortening and as colchicine treatment increases stroke length, the systolic VTI is significantly decreased (−0.25 ± 0.14 mm to −0.32 ± 0.11 mm, $p = 0.013$*) (Fig. 7A). Peak systolic velocity was increased and occurred earlier (0.10 ± 0.01 mm/s to 0.09 ± 0.01 mm/s, $p = 0.009$*) (Fig. 7D, G) while the duration of shortening was not changed (Fig. 7J). Consistent with colchicine

treatment having the greatest impact on early diastolic filling, E VTI (0.15 ± 0.11 mm to 0.20 ± 0.14 mm, $p = 0.059$) and the maximum early filling velocity, E, were increased while the time to peak of early filling was accelerated (0.47 ± 0.04 s to 0.44 ± 0.07 s, $p = 0.039$*). Additionally, the duration of early filling was increased with colchicine treatment likely as a consequence of faster force development and relaxation leaving more time for diastole. Late filling parameters were unchanged with colchicine treatment with both the speed and duration of late filling being unchanged with colchicine treatment.

The work loop mimics the four phases of the cardiac cycle starting with stimulation initiating IVC which generates force isometrically until it rises to the afterload initiating tissue shortening and ejection. The length of the tissue decreases until relaxation starts IVR and in which the force falls below the preload the tissue lengthens. The tissue shortening represents ventricular ejection and the tissue lengthening represents ventricular filling. We were able to distinguish between phases during work loop experiments through the force feedback system. After colchicine, the duration of ejection remained unchanged (Fig. 8B) but accelerated the duration of IVC (0.06 ± 0.01 s to 0.05 ± 0.01 s, $p = 0.004$*) and IVR (0.17 ± 0.06 s to 0.11 ± 0.05 s, $p = 0.006$*) (Fig. 8A, C). The reduced time in the force generating phases allows for an increase in the time available for filling, making it prolonged (0.60 ± 0.05 s to 0.67 ± 0.04 s, $p = 0.002$*) (Fig. 8D). Additionally, using the velocity traces we can separate the time spent in late and early filling. Colchicine did not affect the time spent in late filling but increased the amount of time available for early filling.

4. Discussion

Our ex-vivo cardiac work loop protocol leverages previously established precision-cut myocardial slices to capture working cardiac performance and diastology. Our results demonstrate that microtubule destabilization with colchicine improves the IVR and early-filling performance of myocardial slices while maintaining late-filling and improving systolic function. Previous studies demonstrate that microtubule depolymerization with colchicine reduces the viscoelasticity of cardiomyocytes and myocardium consistently when subjected to physiological strain rates [21]. Colchicine treatment improved mechanical properties across multiple ideologies of heart disease; however, in healthy animals and tissue the mechanical effects of colchicine are less profound [26]. We would anticipate that reduced viscoelasticity with colchicine treatment might appear as a reduction in the EDPVR, but the slope of our late-diastolic stretch is unchanged. This result could appear surprising, but myocardial tissue stiffness is length-dependent and the increased end-diastolic length would increase EDPVR and EDP. Thus, while the post-colchicine work loops show a rightward shift (increased EDL) but no change in EDFLR and no change in EDF (Fig. S1), the end diastolic elastance is reduced. We interpret this as a diastolic benefit associated with stiffness reduction by colchicine. Thus, we feel this data is in good agreement with other recent studies which demonstrate improved stroke work, end-diastolic elastance and early-filling with microtubule-based therapies [30].

The development of this protocol and demonstration that colchicine can increase in cardiac work and early filling characteristics of female Wistar-Kyoto rats is not without limitations. Additional work is needed to determine if there are sex-dependent and disease dependent

affects of colchicine treatment or diastolic performance. An important hallmark feature of diastolic dysfunction is exercise intolerance which may appear with increased pacing frequency in this protocol. Accelerated pacing will reduce the time for diastolic filling and eventually force a merging of the E and A peaks which our preliminary results suggest colchicine may be protective of [31]. Currently our experimental setup is limited to room temperature perfusion which also slows the contractile kinetics of rat myocardium and limits our current experimental protocol to a 1 Hz pacing frequency. Microtubules are known to be more stable at 37C and thus we would anticipate that these results will hold at physiological temperatures which is consistent with microtubule-based therapies showing effects both in-vivo and in isolated cardiomyocytes at room temperature [30,32,33]. Further, microtubules are not easily imaged in our preparation and protein quantification cannot assess microtubule polymerization state and so we were unable to confirm microtubule depolymerization with colchicine. Thus, in theory colchicine could be acting via an effect orthogonal to microtubule depolymerization but we feel this is unlikely as previous studies have demonstrated similar enhancment in contractile kinetics with colchicine which plateau within 60 min of treatment at room temperature [17].

An early symptom of DDx is exercise intolerance. In healthy individuals exercise increases heart rate and stroke volume to cooperatively enhance cardiac output. In DDx, increased heart rate results in an elevated EDP for only marginal increases in EDV [11]. The resultant increased EDPVR, i.e. increased myocardial stiffness, limits the enhancement of cardiac output and thus exercise performance. Our results show that depolymerization of the MTN with colchicine accelerates the isovolumic phases of the cardiac work loop and decreases end diastolic elastance by increasing the end-diastolic length. Thus, we speculate colchicine may improve exercise performance by reducing both the time needed to complete diastole and EDPVR. This would allow for complete diastole at elevated heart rates and enhances the inherent sensitivity of the heart to venous return via the Frank-Starling mechanism. To date, physiologically tolerable doses of colchicine are insufficient to destabilize cardiac microtubules, but more targeted microtubule-based therapies are likely to improve exercise performance.

The performance of healthy myocardium relies heavily on fatty acid oxidation over glycolysis for ATP generation. Thus, it is not surprising that we found traditional KHB solution insufficient to maintain stable isometric twitches for the 90 min required to depolymerize microtubules with colchicine. Cardiac muscle experimental solutions have remained relatively unchanged for the past 100 years, originating with Ringer's, Tyrode's, and Krebs-Henseleit's solutions [34-36].While KHB contains mineral salts, a pH buffering system, and glucose, it lacks the free fatty acids and TCA cycle intermediates critical for long term experimental stability in a continuously pacing system [37]. We selected DMEM/F-12 as a standard media that contains the necessary metabolites but found the calcium concentration needed to be supplemented to maintain isometric twitch force. We chose 1.8 mM calcium to be consistent with previous studies. We also observed that the initial force values are greater in KHB than DMEM/F-12 media possibly driven by forcing the tissues into solely glycolysis. However, the force at 90 min was significantly reduced in KHB compared to DMEM/F-12. For the female Wistar Kyoto rats in this study, we found this

solution maintained stable isometric twitch force and kinetics for hours making it easy to isolate the effect of colchicine on twitch kinetics and tissue work.

DDx is a hallmark of most etiologies of heart failure and arises from molecular contributions of myofilaments, fibrosis, and microtubules. While our results indicate that microtubule destabilization improves diastolic function, future work is necessary to determine if the benefits of microtubule-based therapies differ between types and phases of heart disease. For example, overexpression of tyrosine tubulin ligase (TTL) to reduce microtubule detyrosination in a mouse model of HCM leads to a rightward shift in the PV loop and reduction in EDPVR [30]. In ischemic heart disease, MARK4 mediates an increase in microtubule detyrosination via increased VASH2 activity that can be targeted to improve cardiac performance [38]. As new therapies are developed or repurposed to treat heart failure, for example SGLT2 inhibitors [39,40], a stable ex-vivo system will provide molecular insight into the improvement of diastolic function and patient etiologies likely to benefit from specific interventions. The cardiac slice model is a powerful tool to isolate the cardiac-specific effect of various therapeutic interventions [27].

5. Conclusion

Herein we demonstrate a novel method to perform diastology in working myocardial slice preparations and show that microtubule depolymerization with colchicine improves the performance of working myocardial slices. To maintain slices for the duration necessary to depolymerize microtubules we transitioned from a traditional KHB solution to DMEM/F-12 which exhibited stable isometric twitches for multiple hours. We found that 90 min treatment with colchicine accelerated isometric twitches and reduced passive diastolic tension. Myocardial work was significantly increased by colchicine at constant preload and afterload. Slice diastology demonstrated an improvement in early filling with an increased time for filling. This was driven by an enhancement in the rate of force development and relaxation leading to shorter isovolumic phases. Decreased passive tension led to an increased EDF and reduced end diastolic elastance that likely drives a Frank-Starling mediated enhancement in work. Taken together these results are consistent with in vivo data that demonstrate microtubule-based therapies are capable of improving cardiac performance. Our observation of enhanced filling time and early filling performance further suggests that depolymerization of cardiac microtubules with colchicine could be broadly applicable for the treatment of DDx without compromising systolic performance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

The raw data supporting the conclusions of this article and any code used for analysis will be made available by the authors upon reasonable request.

Non-standard abbreviations and acronyms

BDM	2,3-Butanedione 2-monoxime
COLCH	Colchicine
DDx	Diastolic dysfunction
DMEM/F-12	Dulbecco's modified eagle medium/nutrient mixture F-12
DMSO	Dimethylsulfoxide
EDFLR	End-diastolic force-length relationship
EDPVR	End-diastolic pressure-volume relationship
EDF	End-diastolic force
EDV	End-diastolic volume
HCM	Hypertrophic cardiomyopathy
HF	Heart failure
IVC	Isovolumic contraction
IVR	Isovolumic relaxation
KHB	Krebs–Henseleit buffer
LV	Left ventricle
MTN	Microtubule network
TTL	Tyrosine tubulin ligase
VTI	Velocity time integral

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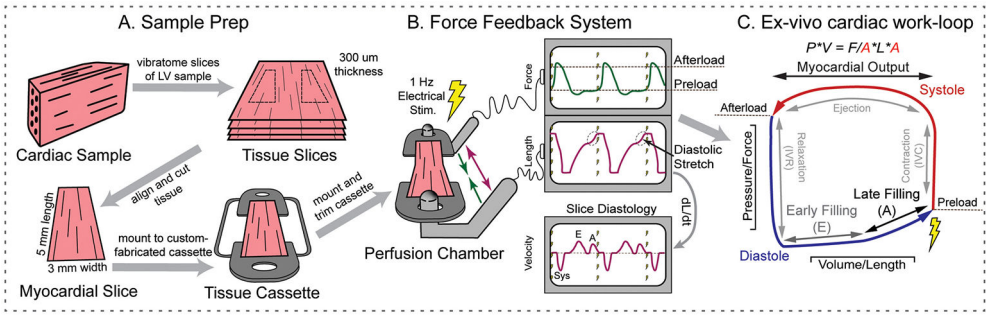

Fig. 1.

Schematic of working cardiac slice preparation. A. Left ventricular free-wall samples from female Wistar-Kyoto rats were sliced 300 μm thick using a vibratome, cut and attached to custom tissue cassette with the fiber orientation parallel to the contractile axis. B. The cassettes were then mounted in a perfusion chamber between a force transducer and length controller, electrically stimulated, and subjected to force feedback to mimic the cardiac cycle. The derivative of the length trace during relengthening provides an analog to trans-mitral doppler and therefore a clinically-analogous grade diastolic performance for the slice. C. Force plotted against length to generate a counterclockwise loop (i.e. a work loop) that is the mathematical analog to the cardiac pressure volume loop ($P * V = (F / A) * (L * A)$). The phases of the cardiac cycle are labelled in the work loop with distinction between early and late filling.

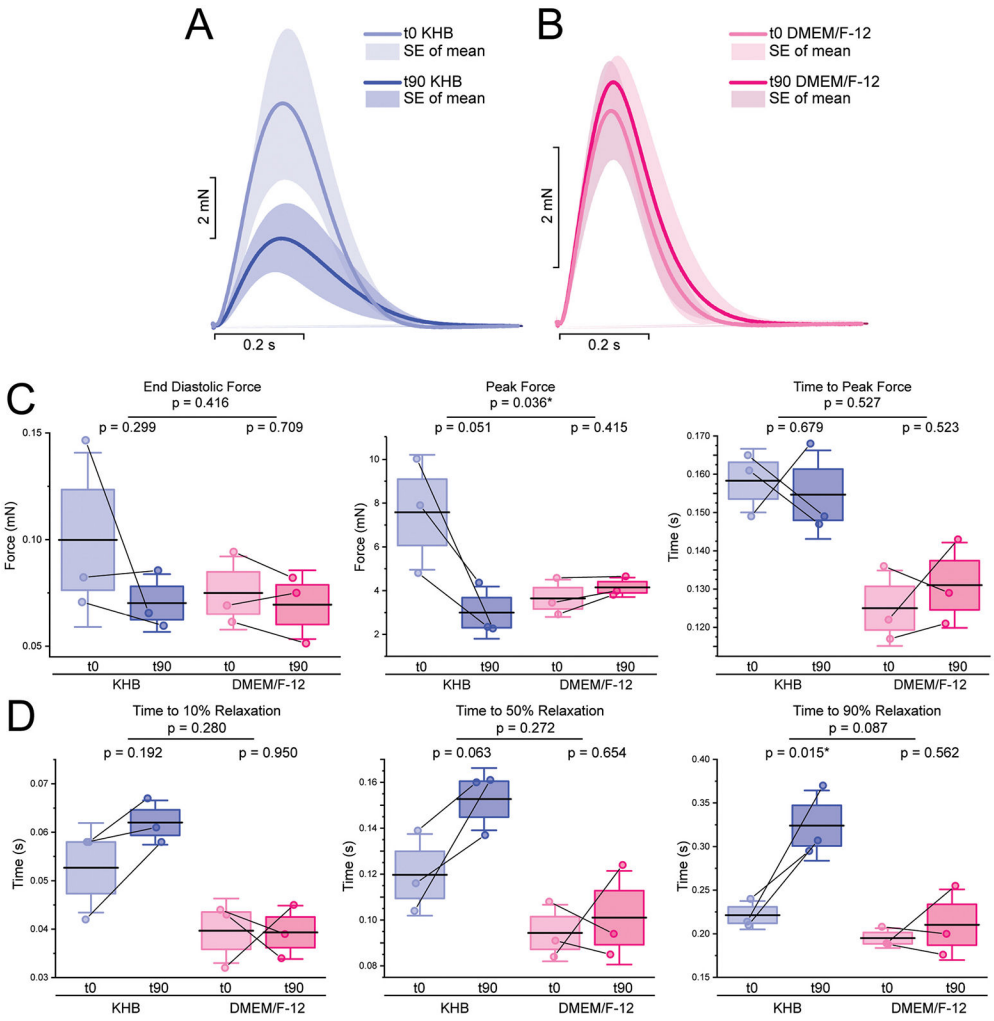

Fig. 2.

DMEM/F-12 Recirculation Preserves Isometric Twitch Force in Myocardial Slices. A. Mean isometric twitches with standard error before and after 90 min in the recirculation chamber with Krebs-Henseleit buffer (KHB). B. Mean isometric twitches with standard error before and after 90 min in the recirculation chamber with DMEM/F-12. C. Box plots with statistics for force parameters. D. Box plots with statistics for relaxation kinetics. Boxes show standard error and whiskers show standard deviation. A paired *t*-test performed within treatment groups (below bar) and two sample t-test performed on net effect of 90 min between treatment groups (above bar). *P*-value less than 0.05* considered significant. $N = 3$, $n = 3$ KHB, n = 3 DMEM/F12.

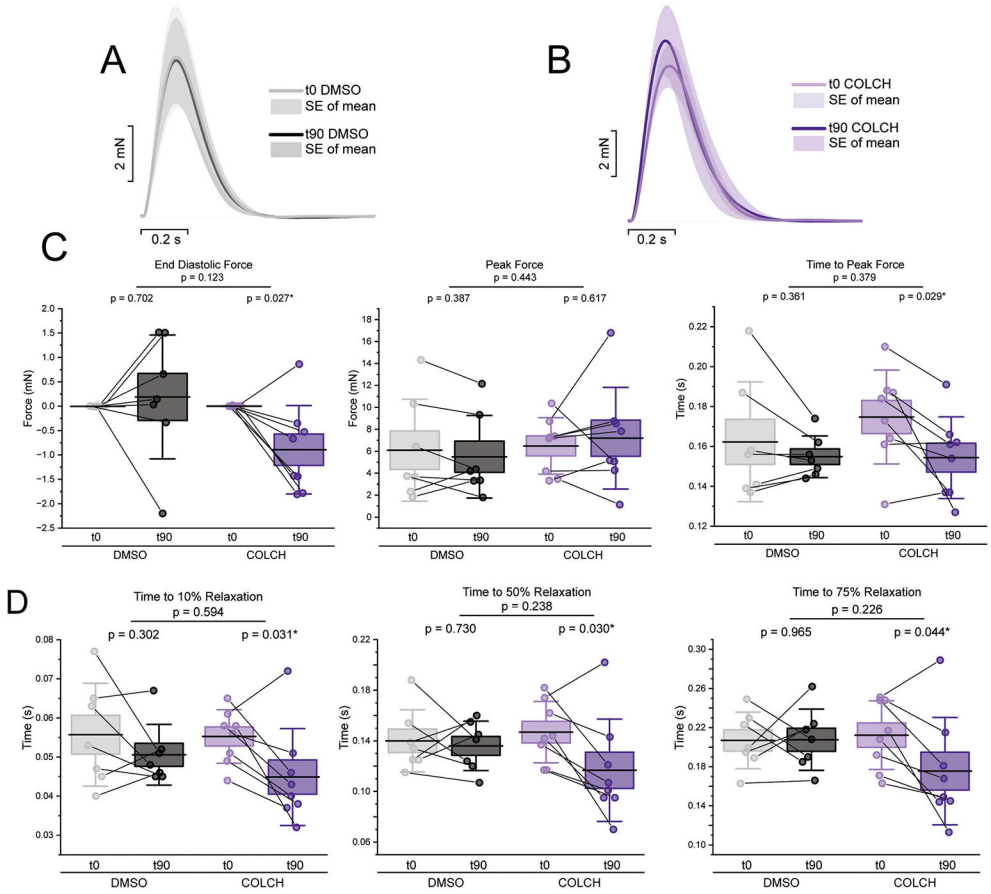

Fig. 3.

Colchicine treatment accelerates isometric twitch force. A. Before and after 90 min treated with DMSO (vehicle). B. Mean isometric twitches with standard error before and after 90 min treated with colchicine. C. Force parameters derived from isometric twitches: end diastolic force, peak force, and time to peak force. D. Relaxation kinetics derived from isometric twitches; time to complete 10 %, 50 %, and 75 % of relaxation. Boxes represent standard error and whiskers are standard deviation. A paired t-test performed for each slice within treatment groups (below bar) and two sample t-test performed on net effect (change between t0 and t90) of 90 min between treatment groups (above bar). P-value less than 0.05* considered significant. $N = 5$, $n = 7$ DMSO, $n = 8$ colchicine.

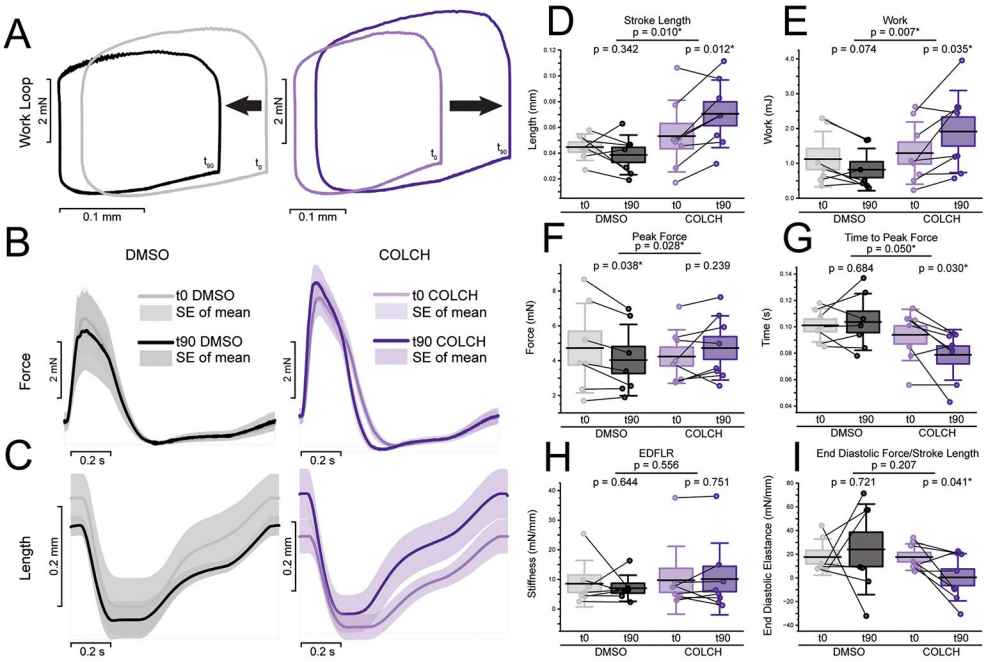

Fig. 4.

Colchicine Increases Work Done by Cardiac Slices. A. Mean force plotted against mean length to generate a force-length work loop before and after 90 min with colchicine or DMSO (vehicle). B. Mean force traces with standard error before and after 90 min treated with colchicine or DMSO. C. Mean length traces with standard error before and after 90 min treated with colchicine and DMSO. D–I. Key parameters of work loops, force traces, and length traces: peak force generation, time to peak, stroke length, work, EDFLR, and End Diastolic Force/Stoke Length with statistics. Boxes represent standard error and whiskers are standard deviation. N = 5, n = 7 DMSO, n = 8 colchicine.

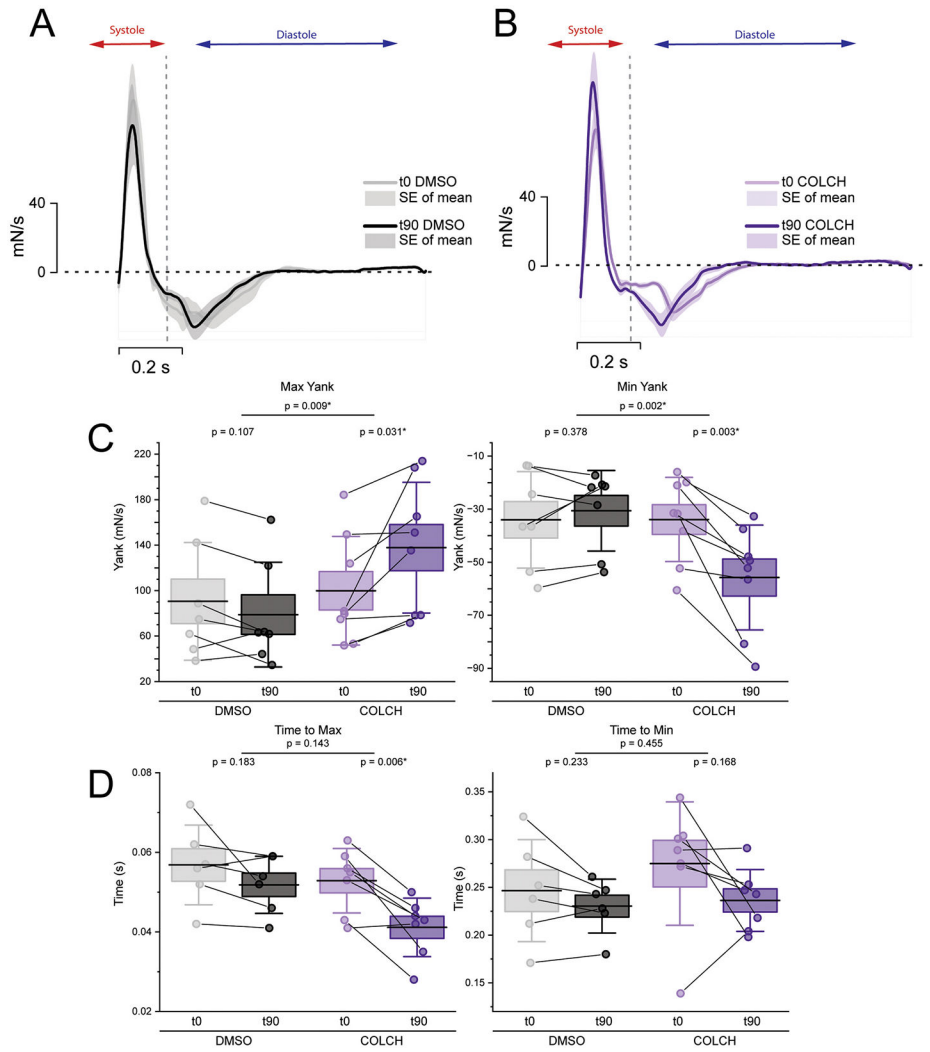

Fig. 5.

Colchicine Increases Myocardial Yank. A. Mean force derivative (yank) with standard error before and after 90 min of DMSO (vehicle) treatment. B. Mean force derivative (yank) with standard error before and after 90 min of colchicine treatment. C. Peak values of yank and statistics on colchicine effect: maximum dF/dt and minimum dF/dt. D. Key kinetic indices: time to maximum and minimum yank. Boxes represent standard error and whiskers are standard deviation. N = 5, n = 7 DMSO, n = 8 colchicine.

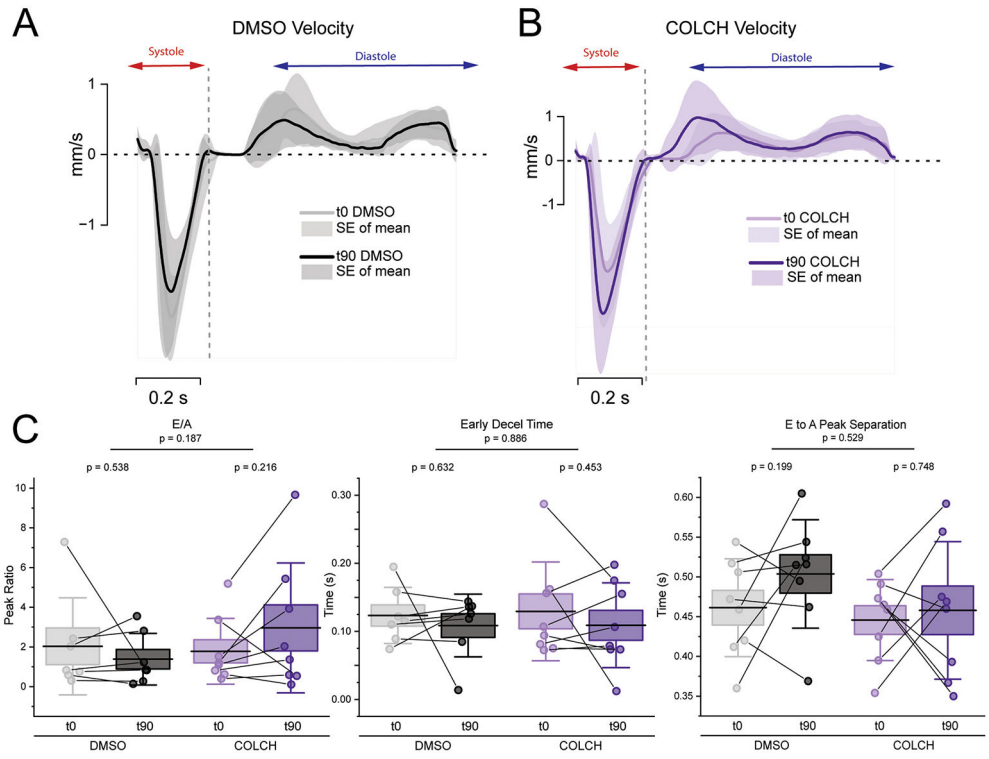

Fig. 6.

Working slice diastology. A. Mean velocity traces with standard error before and after 90 min of DMSO (vehicle) treatment. B. Mean velocity traces with standard error before and after 90 min of colchicine. C. Statistics on common clinically used parameters of diastolic function: E/A, E deceleration time, and E-A peak separation time. N = 5, n = 7 DMSO, n = 8 colchicine.

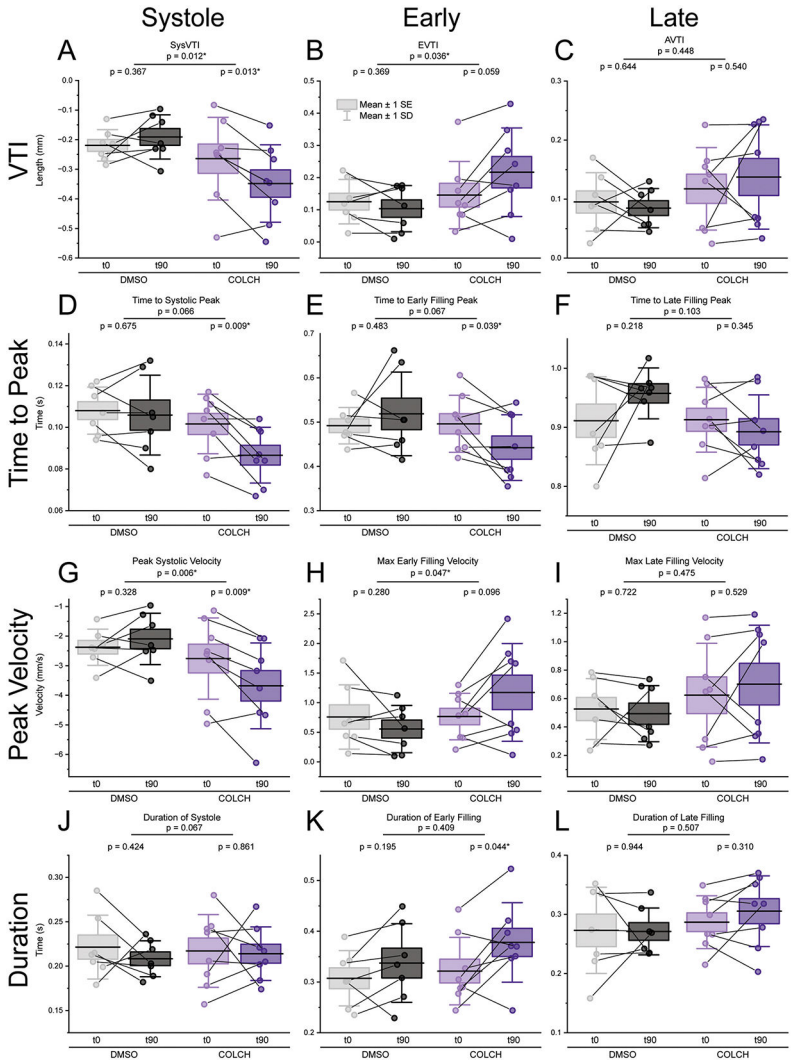

Fig. 7.

Systology and diastology peak quantification table. For systole, early filling and late filling peaks, box plots with statistics between DMSO (gray/black) and colchicine (purple) of VTI, time to peak, peak velocity, duration of phase from mean velocity traces as corresponding to the phases of systole, early filling, and late filling. Boxes represent standard error and whiskers are standard deviation. N = 5, n = 7 DMSO, n = 8 colchicine.

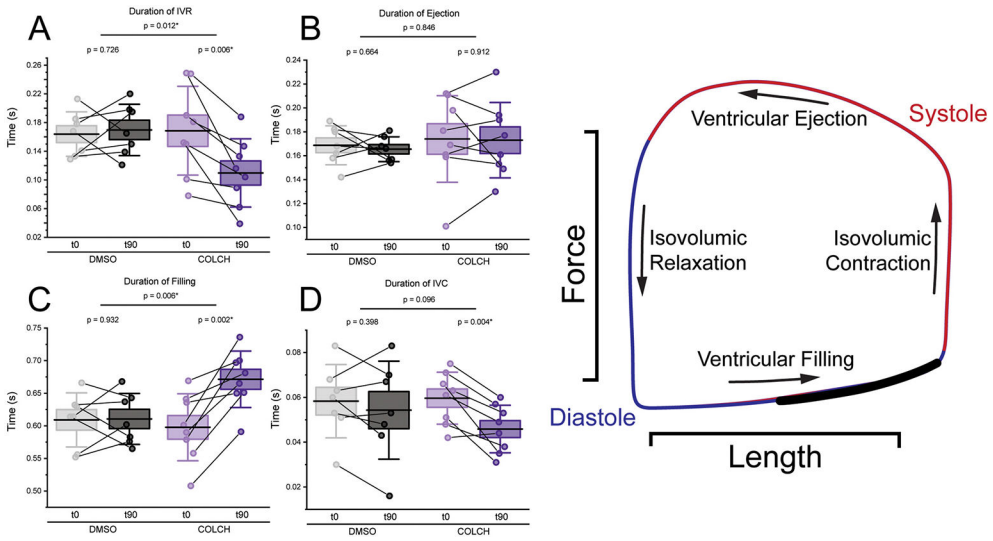

Fig. 8.

Time spent in each phase of the cardiac cycle. A–D. Box plots with statistics for duration of IVC, duration of ejection, duration of IVR, and duration of filling before and after treatment with DMSO (gray/black) or colchicine (purple). Boxes represent standard error and whiskers are standard deviation. N = 5, n = 7 DMSO, n = 8 colchicine.