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The Final thesis

A Systematic Review of the Expected Effectiveness of Electric Microcurrent in Heart Failure Treatment

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TABLE OF CONTENT

1 LIST OF FIGUERS AND TABLES 3

2 KEYWORDS: 3

3 SUMMARY: 4

4 INTRODUCTION 5

4.1 CARDIAC REMODELING: FIBROSIS, INFLAMMATION, EDEMA, AND HYPERTROPHY 5

4.1.1 Cardiac fibrosis: 5

4.1.2 Cardiac inflammation: 7

4.1.3 Cardiac edema: 8

4.1.4 Cardiovascular hypertrophy: 9

4.2 HEART FAILURE TREATMENT MODALITIES TO REDUCE CARDIAC REMODELING 9

5 ENDOGENOUS ELECTRIC FIELDS: PRINCIPLES OF ELECTROPHYSIOLOGY 11

5.1 INJURY CURRENTS 12

5.2 CELLULAR REGENERATION INDUCED BY ENDOGENOUS EF 13

6 MIMICKING ENDOGENOUS ELECTRIC FIELDS TO TREAT CARDAIC REMODELLING 19

6.1 ELECTRICAL STIMULATION AND WOUND HEALING 19

6.2 MIMICKING INJURY CURRENTS TO ACTIVATE ANTI-INFLAMMATORY PATHWAYS 19

6.3 INTRACARDIAC DEVICES USED TO TREAT HEART FAILURE 20

7 LITERATURE SEARCH STRATEGY 23

7.1 AIM 23

7.2 OBJECTIVES 23

7.3 SEARCH STRATEGY AND SCREENING 23

7.3.1 Literature search methods 23

7.4 SELECTION PROCESS 24

7.4.1 Inclusion and Exclusion criteria 24

7.4.2 Selection procedure PRISMA 24

8 DATA EXTRACTION 27

8.1 METHODOLOGICAL APPROACH 27

8.1.1 Data extraction criteria 27

8.1.2 Data analysis 27

9 RESULTS 28

9.1 PARAMETERS OF ELECTRICAL STIMULATION: SUMMARIZED IN TABLES 28

9.2 PRECLINICAL – IN VITRO + IN VIVO STUDIES: SUMMARIZED IN TABLES 30

9.3 CLINICAL TRIALS – CASE REPORTS, TRANSLATIONAL STUDIES AND FIRST PHASE I TRIAL 30

9.3.1 Results: Translation studies 30

9.3.2 Results: case report 31

9.3.3 Results: pilot studies 32

10 DISCUSSION 34

11 CONCLUSION 42

12 REFERENCES 43

13 APPENDIX 48

1 LIST OF FIGUERS AND TABLES

Figure 1: The vicious cycle of cardiac fibrosis. Adapted by A. Piek et al. (6)

Figure 2: Regulation of chronic inflammation in heart failure (2)

Figure 3: Involvement of structural and functional remodeling of cardiac lymphatics in myocardial interstitial fibrosis (24)

Figure 4: Molecular mechanism of cardiac hypertrophy and angiogenesis (26)

Figure 5: Wounding collapses the TEP locally, resulting in an electric field lateral to the plane of the epithelium (44)

Figure 6: Cell movement of an osteoblast induced by an external electric field using physiological current ranges, taken from Levin M. et. al. (55)

Figure 7: Electric fields align collagen molecules; from Becker (1985) (45, 73)

Figure 8: Cascade effects of EF´s – from low to high organizational bioelectric effects; from Levin (74)

Figure 9: Hypothesis of mimicking endogenous electric fields to reduce cardiac remodeling in heart failure (taken from Lujan et. al.) (84)

Figure 10: Schematic system configuration and picture of the actual device from Berlin Heals (IMD = implantable microcurrent device); (MICS = Medical Implant Communication Service) (92)

Figure 11: PRISMA flow chart (99)

Figure 12: Proportionality of microcurrent application and cardiac output (113)

Figure 13: Clinical results taken from Schmitto et. al, intraoperative view: implantation through left thoracotomy, using minimal-invasive surgical technique, LV patch lead was positioned on the LV epicardial surface and fixated, both LV and RV leads were connected to the C-MIC generator (A-D) (94)

Figure 14: Angiographic view (A) and postoperative computed tomography scan (B) (94)

Figure 15: Results from the first single-arm, non-randomized pilot study, from Kosevic (92)

Table 1: Eligibility criteria

Table 2: Type of microcurrent used for electrical stimulation

2 KEYWORDS:

Microcurrency, Microcurrent, Electrical stimulation (ES), Electrical current (EC), Direct current (DC), Electric field (EF), Applied electric field, Endogenous electric current/field

3 SUMMARY:

Background: Heart failure is a complex disease of the heart´s cellular and molecular components, lacking efficient long-term treatment possibilities and reducing the quality of life of many patients. Today, we know a lot about the pathological changes that occur, but little is known about the complex interactions that guide those regenerative processes after cellular damage. Its mediators are possibly under the influence of endogenous electrical fields and, in particular, injury currents that are established immediately upon membrane disruption. These electrical signals may enable intercellular, bidirectional transport of ions, metabolites, second messengers, and other smaller molecules during the process of regeneration. Endogenous electric fields may introduce, guide, and regulate the regenerative cascade in cardiomyocytes after injury.

Purpose: This systematic review examined the effects of exogenous subthreshold microcurrent stimulation on cardiomyocytes/ progenitor cells, or the whole heart, and investigated whether these effects can improve cardiac function in failing hearts.

Methods: Using the keywords listed below, online databases like Medline, Google Scholar, Epistemonikos, Scopus, and Cochrane Databases were searched for abstracts or full articles that used applied subthreshold electrical stimulation on cardiomyocytes for therapeutic purposes.

Results: 21 studies, including full text articles and abstracts, using exogenous subthreshold microcurrent stimulation on cardiomyocytes have been analyzed. The study types involved were preclinical in vitro trials, small case preclinical animal trials and non-randomised clinical phase I pilot studies. Different parameters, intensities, and durations of microcurrent application have been used in all studies reviewed. Preclinical in vitro studies have shown that exogenous subthreshold microcurrent application modulates the extracellular matrix, the inflammatory cascade, as well as promotes ATP production and leads to cardiogenesis if stimulated. Preclinical in vivo results have also confirmed that microcurrent modulates the extracellular matrix and the inflammatory cascade but also demonstrated an influence on angiogenesis, influencing capillary density. Clinical phase I trials have shown short-term rapid improvements in clinical ejection fraction and left ventricular end-diastolic diameter.

Conclusion: The evidence of effects of microcurrent application on cardiomyocytes, their progenitors, or the whole heart is convincing and promising to justify further research as an innovative treatment approach in regenerative medicine.

4 INTRODUCTION

It is currently estimated that the prevalence of heart failure (HF) is at 1-2% of the general adult population, which includes 64.3 million people worldwide. Furthermore, it is currently appraised that numbers continue to rise, especially because of the growing and ageing population living a typical Western lifestyle. This evolving trend shows that despite ongoing improvements in survival rates after diagnosis, which have led to stable mortality rates from the 1990s onward, that the burden is still demanding and new and innovative treatment approaches are needed to cope with the increasing number of patients with chronic HF and especially with heart failure with preserved ejection fraction (HFpEF)(Leftventricular ejection fraction; LVEF at 50%) in the near future (1, 2). Furthermore, routine practices by many cardiology societies have generalized heart failure treatment despite the individual clinical status of the patient or the etiology of heart failure nor the exact stage of heart failure. In particular, the advanced heart syndrome is challenging, showing morbidity compression and reduced quality of life (QoL). The progressive nature of the syndrome, characterized by changing pathophysiology, influenced by various comorbidities and treatments, shows extreme difficulties in providing the most effective treatment for certain stages of the disease (3).

4.1 Cardiac remodeling: fibrosis, inflammation, edema, and hypertrophy

4.1.1 Cardiac fibrosis:

HF is a complex syndrome in which the clinical manifestations result in a complex interplay of dysfunctions and changes to the heart´s cellular and molecular components and to mediators that drive homeostatic control. The main term to describe various effects during chronic heart failure is cardiac remodeling, which encompasses effects like: myocyte hypertrophy, necrosis, apoptosis, fibrosis, uncontrolled inflammatory processes, and chronic edema. One of the key concepts is cardiac fibrosis post-MI injury (4, 5). The term "fibrosis" is described as: "the excessive deposition of ECM proteins in parenchymal tissues, and typically reflects inappropriate or unrestrained activation of a reparative program" (4).

The response of the heart to induced cardiac remodeling is due to the increased ventricular wall stress, afterload (increased pressure load), as well as the preload (volume load), which all belong to the main contributors of HF (5). One of the most prominent cellular actuators are activated fibroblasts, also called myofibroblasts (MFB), which can contract and produce ECM-components. Especially in stressed and infarcted hearts, fibroblasts turn into myofibroblasts.

Inflammatory cells (macrophages, neutrophils, and lymphocytes) can induce their activation by producing cytokines (tumor necrosis factor alpha (TNFα), interleukin (IL)-1b, and IL-6 (6)), chemokines (CXCL-1 (7)), and neurohumoral activators (angiotensin II/AT1 axis and aldosterone (8)) and many more (9-11). The most potent factor for MFB transition is tumor growth factor beta (TGF-ß*)* (5, 12). TGF-ß changes the protease/anti-protease balance into a matrix-preserving phenotype by stimulating protease inhibitors, for example, plasminogen activator inhibitor (PAI)-1 and tissue inhibitor of metalloproteinase (TIMP)-1 (13, 14) by hampering the synthesis of matrix metalloproteinases (MMP) (15, 16). MMPs, in turn, are synthesized by myofibroblasts and released as inactive zymogens. After activation, MMPs can cut up the ECM proteins (5). In turn, TIMPs, which are also released by myofibroblasts, act as a repressor for MMP s and inhibit their transcription from occurring. This complex functional interplay of the ECM is mainly determined by myofibroblasts (6). A hallmark of cardiac fibrosis is the secretion of collagen I and III, which have different properties. Type I is thicker and stiffer, while type III is more compliant. Both types are secreted by activated fibroblasts and myofibroblasts and lead to cardiac fibrosis (5-7).

The effects of cardiac remodeling and cardiac fibrosis are directly linked to HF and cause heart rhythm disturbances (due to atrial fibrosis, remodeling of ion channels and perivascular fibrosis), reduced oxygen diffusion (due to hypertrophy), and reduced systolic and diastolic function (due to myocardial stiffening). These effects will inevitably lead to cell death (4-7).

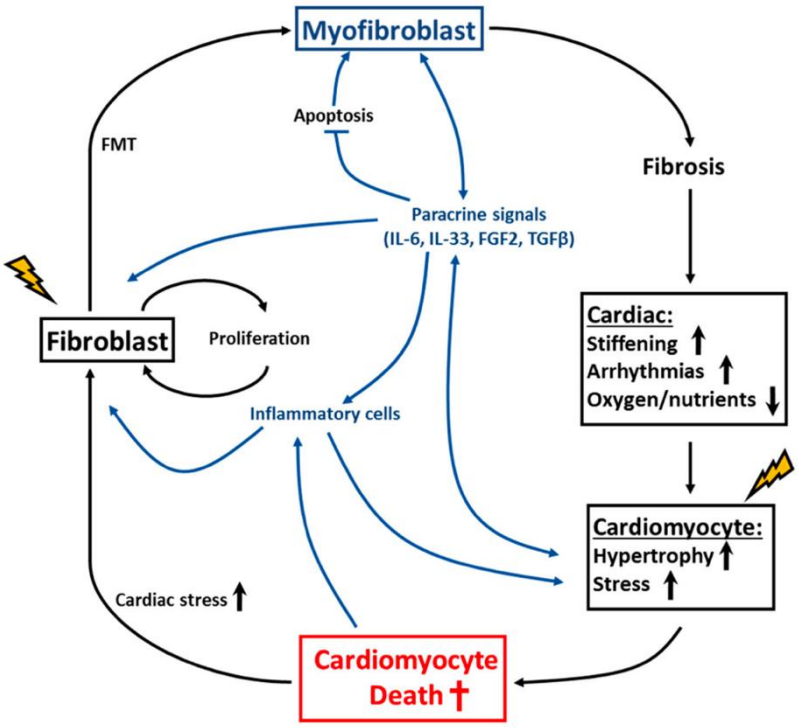

Figure 1. The vicious cycle of cardiac fibrosis, a simplified scheme, from A. Piek et al. (6)

4.1.2 Cardiac inflammation:

Elevated inflammatory biomarkers have been detected in many different cardiac disease entities, but therapeutic interventions to reduce the proinflammatory cytokines in the chronic setting remain controversial. This is mainly because physiological inflammation cannot be distinguished from pathological inflammation. Physiological inflammation is a process that remains important for homeostatic and reparative mechanisms and targeting physiological inflammation incorrectly can lead to worse outcomes. Nevertheless, accentuation, prolongation, or expansion of the postinfarction inflammatory process led to worse remodeling and dysfunction of the myocardium (2, 17). Figure 2 illustrates the interplay of cardiac inflammation (2).

Various inflammatory signals are involved in reducing and stopping the ongoing inflammatory cascade. Important are toll-like receptor signaling (18) and downmodulation of cytokine responses (2) and termination of chemokine signals (19). These processes may be guided by the concerted action of multiple suppressive pathways that prevent the extension of injury and protect the heart from adverse remodeling. Important suppressors of inflammation are interleukin-10 (20), members of the TGF-family (21), and pro-resolving lipid mediators (such as lipoxins, resolvins, and protectins) (22).

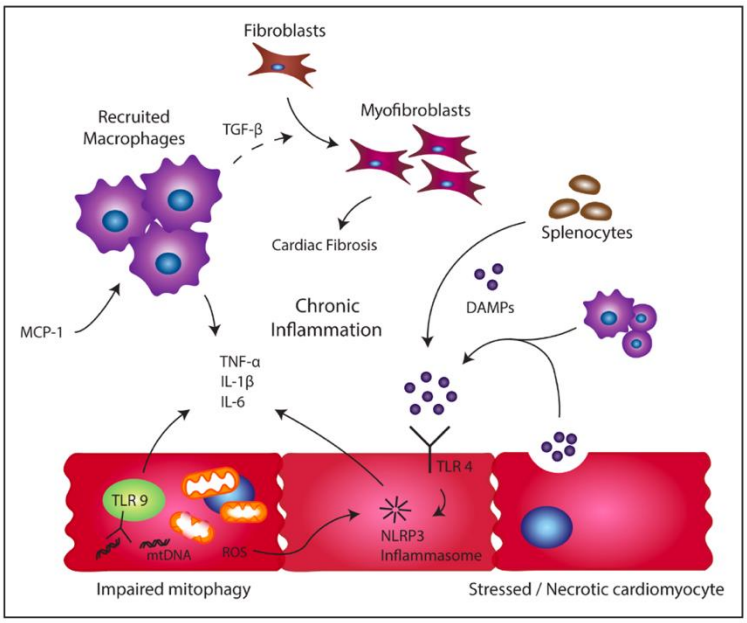

Figure 2: Regulation of chronic inflammation in heart failure. Adapted from the work of S.Dick et al. (2)

4.1.3 Cardiac edema:

Another hallmark influencing cardiac function in the process of cardiac remodeling is myocardial fluid homeostasis. It is primarily determined by the fluid filtration rate from the coronary microvasculature exchange vessels to the interstitium and the lymphatic clearance rate of fluid from the interstitium. Cardiac edema occurs when the rate of fluid filtration exceeds the rate of lymphatic clearance, or vice versa, and fluid accumulates in the interstitium (2). It is also known that chronic cardiac edema induces the deposition of interstitial collagen, causing interstital fibrosis as well as reducing systolic and diastolic functions while raising the stiffness of the left ventricle (LV) (23). This concludes that chronic edema is especially inducing cardiac remodeling. The myocardial microvascular–interstitial–lymphatic structure is of major importance with regards to the compensatory LV chamber compliance to preserve its functionality when challenged with acute edema. This self-perpetuating pathology eventually results in chronic edema and inflammation, which further stimulates fibroblasts to turn into myofibroblasts, inducing a pro-fibrotic response resulting in myocardial interstitial fibrosis that continually restricts lymphatic uptake. Ultimately the elevated interstitial fluid pressure and the cardiac interstitial fibrosis contribute to raising left ventricular stiffness and cardiac dysfunction (24).

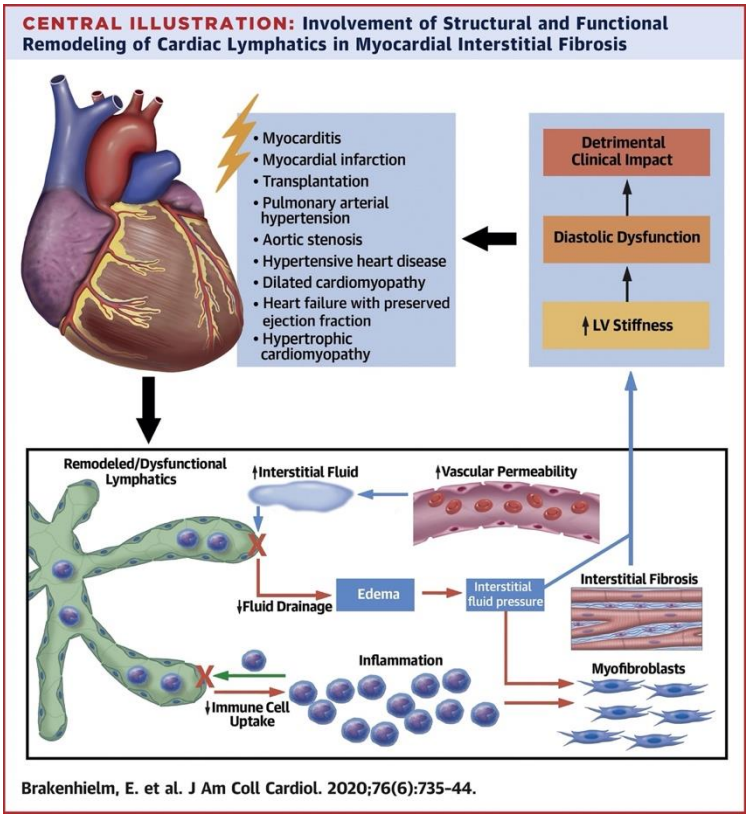

Figure 3: Involvement of structural and functional remodeling of cardiac lymphatics in myocardial interstitial fibrosis (24)

4.1.4 Cardiovascular hypertrophy:

During the process of heart failure, we can distinguish between adaptive processes of the heart and maladaptive ones that lead to decompensated heart failure. One of the adaptive processes is cardiac hypertrophy. In contrast to physiological hypertrophy, which is accompanied by angiogenesis, pathological cardiac hypertrophy induced by hemodynamic overload leads to myocardial ischemia and cardiac dysfunction without angiogenesis (25, 26). This is known as "hibernation" (27). In murine models, induced hypertrophic hearts showed increased expression of vascular endothelial growth factor (VEGF) and angiopoetin 2 genes in the adaptive phase of cardiac hypertrophy and in the chronic maladaptive phase. Following the activation of the *Akt1* gene, angiogenesis was significantly reduced by a decoy VEGF receptor, which led to decreased capillary density, contractile dysfunction, and impaired cardiac growth (28). Thus, both heart size and systolic function are angiogenesis dependent, and disruption of coordinated tissue growth and angiogenesis in the heart contributes to the progression from adaptive cardiac hypertrophy to decompensated heart failure (29) (see figure 4).

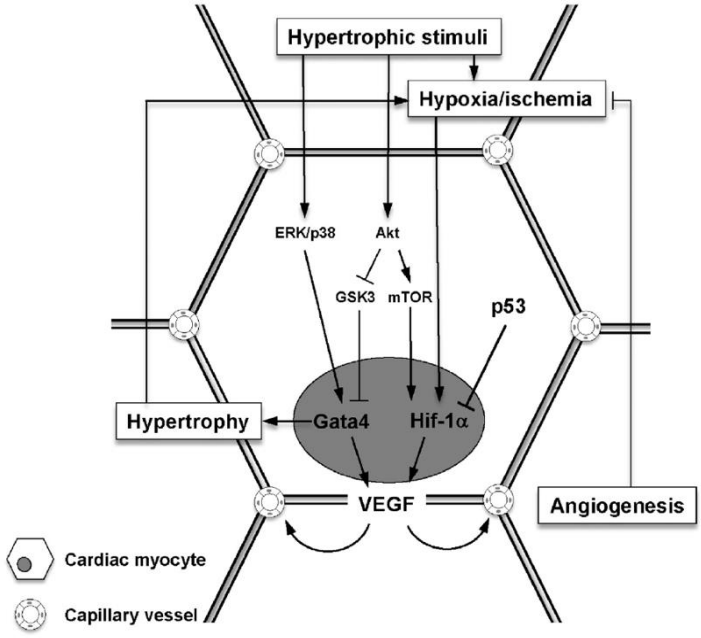

Figure 4: Molecular mechanism of cardiac hypertrophy and angiogenesis (26)

4.2 Heart failure treatment modalities to reduce cardiac remodeling

Whether cardiac fibrosis can be sufficiently reversed currently depends on the etiopathophysiology of fibrosis, the size of cardiac remodeling, and the biochemical effects involving the ECM.

In many cases, fibrosis is seen as a single disease entity and treated like a primary fibrosis, but one can state today that cardiac fibrosis is an often-occurring pathological reaction to many cardiac diseases, and most of the time it represents an adequate secondary reparative answer to a primary damage. Repairing cardiomyocyte necrosis by inducing a fibrotic tissue response is most likely because of the reduced regenerative potential of the human heart. The highest chances for successful treatment of cardiac fibrosis are by targeting early evolving interstitial or perivascular fibrotic processes (3). Analyzing many different treatment trials using pharmacological approaches like lisinopril (30, 31) or valve replacement and left ventricular assist devices (32–34) has not brought strong clinical evidence of fibrotic reversal. Targeting TGF-ß with direct inhibitors has shown limited clinical use due to increased aortic rupture in mouse models as well as induced valvular lesions (35, 36).

Clinical trials involving IL-1 inhibitors have not shown sufficient favorable effects on cardiac efficiency (37). Inhibiting the LOX enzyme system can decrease the degree of myocardial collagen formation and collagen cross-linking in combination with reduced LV dilation and preserved cardiac contractility in MI using mouse models (38).

It is important to understand that targeting single anti-fibrotic factors most often does not lead to desired clinical because single factor treatment destroys the context-related importance of fibrotic remodeling and the pathophysiological heterogenicity of myocardial disease, which cannot be seen as a primary fibrotic disease (3).

Also, altering myocardial edema formation initiates a cascade of events. Increased removal of fluids disturbs the balance of mechanical properties like hyaluronan, which can be removed from the interstitium, thereby inducing fast fluid removal. Additionally, it is known that the functional adaptation during increased fluid accumulation enables the chronically edematous heart to maintain LV chamber compliance when challenged with acute edema (24). Therefore, it represents a protective and preserving adaptation for cardiac function over a wide spectrum of interstitial fluid pressures (39).

Revising the literature concerning treatment modalities that can reverse cardiac remodeling, it becomes evident that we still lack a detailed understanding of how cellular and non-cellular interaction influence the remodeling process. A. Mouten et al. identified certain knowledge gaps regarding the possible utilization of heterogeneity across different cell subtypes to efficiently develop useful interventions, as well as coupling molecular and cellular phenotypes to cell and tissue physiology to reduce the knowledge gap.

Furthermore, it is still unclear how to efficiently aim for endogenous and exogenous signaling pathways that are needed to interpret the outcomes in the relevant clinic. In addition, it is important to identify the different molecular phases and cell physiological changes post-cardiac injury, starting from the shift of normal homeostasis to the inflammatory phase and continuing with the post-inflammation phase to the phase of cellular repair. The definite stage post-cardiac injury describes a homeostatic-like terminal point which forms a new homeostatic equilibrium after the injury (40).

Despite the present knowledge gap regarding treating cardiac remodeling, all approaches using optimal guideline-directed medical therapy (GDMT) have shown no clinically significant impact. Innovations in device-related therapies are seen as a major change in heart failure treatment in recent decades. Novel therapies are needed to target structural or molecular cardiac dysfunctions that are not directly responsive to pharmacological interventions. Complementary device-related therapies might bridge a current therapeutic gap for progressive disease despite GDMT (41).

5 ENDOGENOUS ELECTRIC FIELDS: PRINCIPLES OF ELECTROPHYSIOLOGY

Everything started with the experiments of Galvani in the late 1700s. He elicited muscle contractions from the preparation of frog nerves. His major achievement was recognizing the existence of bioelectricity. He induced twitching of the frog leg by touching it with the sliced end of the sciatic nerve from the opposite leg (42, 43). Additionally, experiments by the German physiologist Emil Du-Bois Reymond showed the existence of static electric fields (EFs) between the right and left forefinger of 2-10 millivolts (mV). Later, more advanced research revealed voltage gradients between cells induced by ion separation, which are not just physiological signs of standard metabolism or classical membrane potential, but specific and instructive signals for key processes during a range of events, from embryonic development, intracellular short-range communication to adult wound healing (44, 45).

In recent years, one can observe a paradigm change regarding the effects of EFs on cell biological processes. Endogenously produced EFs produce gradients of voltage within tissues which influence the cellular response to tissue repair (46-49), embryological development (50-52), stem cell differentiation (53, 54), nerve growth (55), cell migration (53, 55, 56), and proliferation and apoptosis (57).

5.1 Injury currents

Individual cells maintain an electrical potential across their intact plasma membrane. This transepithelial potential (TEP) is produced by specific ion channels which segregate certain ions. The inside of the cell has a net negative charge relative to the outside, which has a net positive charge. This results in a significant potential difference across the highly electrically resistant intact epithelial membrane. The TEP has a strength of tens of millivolts and can therefore be seen as a biological "battery". This principle applies to all ion-transporting epithelia (e.g., skin, or cornea). Once this membrane is injured in an individual cell, an inward injury current will be produced in which positively charged ions enter the cytoplasm. This current leak leads to a collapse of the TEP at the wound. The TEP is, however, not affected further radially where ion properties remain intact. Eventually, positive ions will leak out of the wound because ion pumps laterally to the wound will keep trying to maintain the TEP. This leads to an outward injury current with a lateral voltage gradient (an electric field) oriented parallel to the epithelial cell sheet. The vector of the electric field EF is pointing towards the wound. The current is carried by charged ions, mainly Na^+ and Cl^-. The voltage difference per unit distance characterizes the electrical field (in a cellular context, in millivolts per millimeter (mV per mm)). The size and strength of the EF depends on the tissue resistance (R_{tissue}).

The direction of current flow is the direction of the flow of positive ions. The wound is therefore more negative than the distal regions within the tissue. The injured side is therefore the cathode of the electric field. The EF strength (mV/mm) inversely decreases with distance from the wound as the TEP (in mV) increases. The strength of these electrical signals is much less comparable to a neuron's depolarization (which is 1-2 V/mm). EFs with direct current (DC) that play physiological roles in regeneration and even in development are three or four orders of magnitude less than this (around 1–100 mV/mm) (see figure 6).

These injury potentials, which generate endogenous electric fields, remain at the wounded epithelium for hours or even days in areas of active cell metabolism and development. They are essential key regulators to guide appropriate cellular behavior during tissue morphogenesis and regeneration. Additionally, one can observe that the endogenous EF, which is a vector, influences the non-vectorial chemical diffusion of charged molecules. To conclude, whenever an endogenous EF arises and asserts an effect on charged molecules in vivo, it will regulate the spatial pattern of the resulting chemical gradient. This direct influence on the extra cellular matrix introduces endogenous EFs as a key regulator for complex cellular adaptation mechanisms that happen during cellular stress (44)

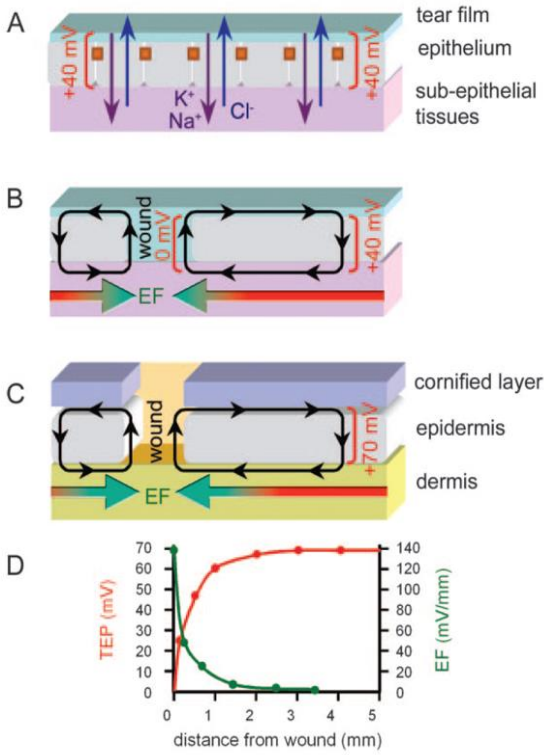

Figure 5: Wounding collapses the TEP locally, resulting in an electric field lateral to the plane of the epithelium (44)

5.2 Cellular regeneration induced by endogenous EF

Having introduced this concept of injury currents, I will now put this concept into context and describe the single bioelectric effects of those EF which arise physiologically as dynamic electrical signals during cellular regeneration. Endogenous EFs play a mediating role during intracellular processes across cytoplasmic membranes but are also important extracellularly, being guidance cues for migrating and proliferating cells. It is important to mention that many cell types migrate directionally in an electric field. This phenomenon is known as electrotaxis or galvanotaxis (see figure 7) (55). Experiments have shown that fibroblasts and many other cell types migrate to the cathode when an externally applied field strength is around 0.1–10 V/mm (58). An exception is the human vascular endothelial cell, which migrates to the anode (44). The factors affecting electrotaxis are the strength of the EF and the cell subtype (55). When the electrical stimulus was applied with a reversed polarity compared to the endogenous field, the default wound-healing cues failed to drive wound closure. It is remarkably striking that the wound even opened up. One interpretation of this observation is that the endogenous physiological EF belongs to the top hierarchy of the regeneration cascade when the wound healing process is initiated because it can override pre-existing chemical and physical wound-healing cues (57).

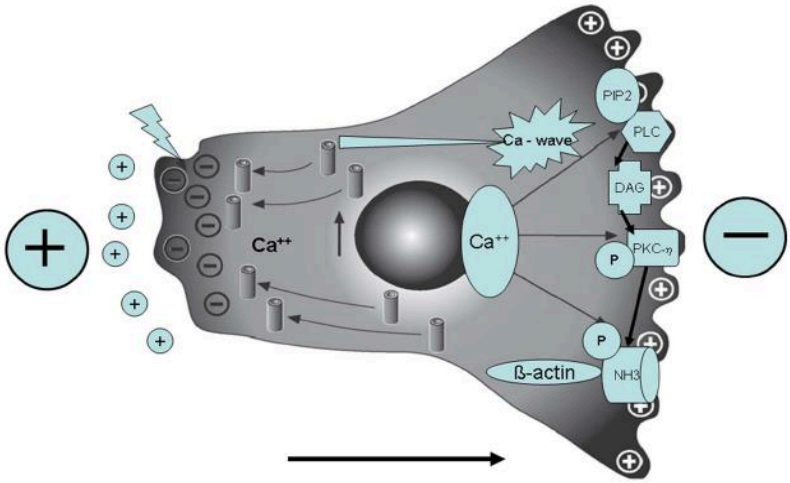

Figure 6: Cell movement of an osteoblast induced by an external electric field using physiological current ranges, taken from Funk. et. al. (55)

Especially during cardiac remodeling, which encompasses effects like myocyte hypertrophy, necrosis, apoptosis, fibrosis, increased fibrillar collagen, and fibroblast proliferation (59). These pathological processes happen at a cellular level. Therefore, its mediators are under the influence of endogenous EF and, in particular, injury currents, which are established immediately upon the destruction of a cell membrane and are of major importance in triggering the process of regeneration.

During epithelial rupture, the potential differences create a "short-circuit" across the whole epithelial sheet. This wound-induced EF lasts for several hours to days and regulates cell behaviors within 500m to 1mm of the wound edge. In his review, McCaig et al. (2005) state: "in evolutionary terms, membrane resealing to close an electrical leak is among the most primitive activities that cells undertake." Perhaps both single cells and sheets of cells use the instantaneous electrical signal induced by injury to seal a membrane and to close a wound, respectively "(44). The signal then fades when the complete covering of the wound by the epithelium has taken place (44). Regarding the time course of corneal epithelial wound healing *in vivo*, Kucerova et al. showed that the electric field first triggers only the initial signals after wounding, like planar polarization of the cells, while other factors like growth factors and others take over later in the process (60).

Zhao et al. recently shed more light on the mechanism of how electric stimulation triggers wound healing by analyzing the translation of electrical signals into the biochemical cascade. Their research identified that an electrical stimulus induces the activation of Src and inositol–phospholipid signaling, which polarizes in the direction of cell migration in the corneal tissues of rats.

Additionally, they found that genetic interference of phosphatidylinositol-3-OH kinase-g (PI(3)Kg) reduces electric-field-induced signaling and terminates directed movements of the healing epithelium in response to electric signals. In contrast, deletion of the tumor suppressor phosphatase and tensin homolog (PTEN) increases signaling and electrotactic effects. Summarizing these data shows that PI(3)Kg and PTEN mediate the electrical response to coupled intracellular responses via ion channels to induce wound healing by controlling electrotaxic cell migration (61).

These examples illustrate the fast onset of electrical guidance during the regeneration process and point out that EF bridges the information gap between short-range molecular action and long-term hormonal action like endocrine distribution via blood flow and long-range actions of the nervous system.

This is particularly important because myocardial cells are equipped with gap junctions, which can amplify the electrical signal and thus many more cells are involved in the signal circuit (62). Small molecules, such as serotonin, can be driven through connexin gap junction channels by DC EF. They enable intercellular, bidirectional transport of ions, metabolites, second messengers, and other molecules smaller than 1 kD. In this context, Levin et al. (2006) have identified serotonin as a "second" messenger (63). Also, Axel T. Esser et al. developed a mathematical model which quantitively supports the plausibility of electrophoretic control of morphogen movement through gap junctions during early left-right patterning during embryogenesis (64)

Rajnicek et al. showed profound nerve growth and guidance effects of applied EFs with strengths as low as 7 mV/mm. Growth cones (the motile tips of growing nerves) of dissociated Xenopus embryonic spinal-cord neurons (the predominant in vitro model of chemotropic axon-guidance studies) rapidly and dramatically changed direction to migrate towards the cathode in response to these EFs. They also branched more frequently towards the cathode, migrated more rapidly towards the cathode and advanced relatively slowly in the direction of the anode (65).

One can state that nerve supply is a major contributor to regeneration (66). Studies which have shown that neurites are galvanotactic (67) and that applied EFs produce a striking hyperinnervation have led researchers to postulate that blastema currents influence regeneration by attracting migratory neuronal cells to the regeneration focus (68). Today, we know that exogenous currents can cause regeneration in peripheral as well as in central nervous tissue (69).

Another important mechanism is the influence of ion fluxes on programmed cell death, or apoptosis. In particular, K^+ channels and membrane hyperpolarization are paramount (70). It is now widely accepted that apoptosis is a required component in the early stages of regeneration (71). Additionally, ion flows have been involved in cell differentiation, which is an important aspect in cell tissue regeneration of complex structures as well. Recent findings have implicated the calcineurin pathway in linking Kir2.1-mediated hyperpolarization with differentiation in human myoblasts (72).

The most important aspect of cardiac remodeling is fibrosis and increased fibrillar collagen deposition by myofibroblasts. Fibrosis results in stiffening of the heart, conductivity problems, and reduced oxygen diffusion, and is associated with diminished ventricular function and arrhythmias (6). In 1985, Becker discovered that the alignment of collagen molecules (representing piezoelectric molecules) due to mechanical stress induces an electrical vector. In addition, the electrical current stimulates cell growth and tropocollagen formation (45, 73) (see figure 3). This observation could link the increased fibrosis and collagen deposition during cardiac remodeling to the hierarchy of physiologic effects of endogenous EF.

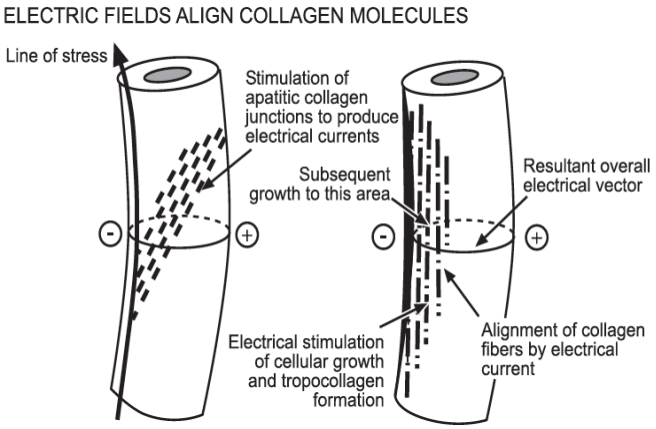

Figure 7: Electric fields align collagen molecules; from Becker (1985) (45, 73)

Bioelectrical signals have the intrinsic property of governing electric fields. They can mostly be understood as an epigenetic mechanism because physiological networks can control and create order in the absence of changes in DNA, RNA, or protein expression. Most likely, these EFs can be seen as an evolutionarily-ancient model of living systems, derived from basic physics, which assures that injury automatically provides cells with a vector cue indicating the position of the damage. An interesting and key consequence of multi-scale control of bioelectrical signals is their capability to act as "master regulators" by activating coherent downstream morphogenetic cascades (74).

To summarize the bioelectric control mechanisms of endogenous EF, I will present a schematic summary from Levin et al. that visualizes the organizational level as well as the cascade and downstream effects that are primed and guided by EFs (see Figure 9).

He postulates that endogenous EFs are one of the most important epigenetic modulators of cell behavior and involve feedback loops, long-distance communication, polarity, and information transfer over various cellular sizes, cell sheets, organs, and even appendages. Looking at living biological systems, the vast majority of all components are electrically charged, and today we know that there is a localized physiological electrical gradient distributed over the organ tissues, which is expressed in a current density in the order of several hundred A/cm^2, responsible for the electrotaxis of cells and molecules.

So, it is obvious that internal and externally applied EFs must have an effect on charged components of an organ. It is also obvious that the effect of an electric field affects all charged components and that the biological response to EFs is extremely complex and diverse and cannot be described by an isolated effect on one component. It is rather dependent on the spatial-temporal occurrence of the field (74, 75).

The schema in Figure 9 summarizes the effects of endogenous EF on various stages, starting from its source and origin and leading to large-scale morphogenetic processes. The ion channels, gap junctions, and the break in the epithelium, which I mentioned at the beginning of this chapter, generate bioelectric signals. Following these signals, which are present as variances in transmembrane potential, pH gradients, ion flows, or electric fields, one can distinguish cell-autonomous processes (seen in purple) as well as non-cell-autonomous processes (seen in pink) or a combination of both. The transduction happens via different proximal epigenetic operations, which are voltage-sensing domains on certain proteins, gating of morphogen transporters, and flow of specific ions (especially Ca^{2+}). The green color on the 3rd stage shows electrical effects, and the yellow color illustrates a biochemical impact because of ion identity. The following secondary response, which was guided by epigenetic signals, is now translated into genetic pathways like NF-kB, Notch, PTEN, Slug/Sox10, and many more (see Figure 9). The genetic influence now guides the downstream signals which control alterations in cell number (proliferation, apoptosis), cell position (migration, orientation of cleavage plane) and cell type (differentiation and de-differentiation). The final effect stage influences morphogenetic processes like blastemas, embryonic fields, and polarity decisions (74, 75).

Concluding, the multitude of cellular effects which I have introduced above and many more I cannot mention due to space constraints show the importance of endogenous EFs in the process of cellular regeneration of injured cells and provide useful links which can be applied to the pathophysiological mechanisms of cardiac remodeling in HF.

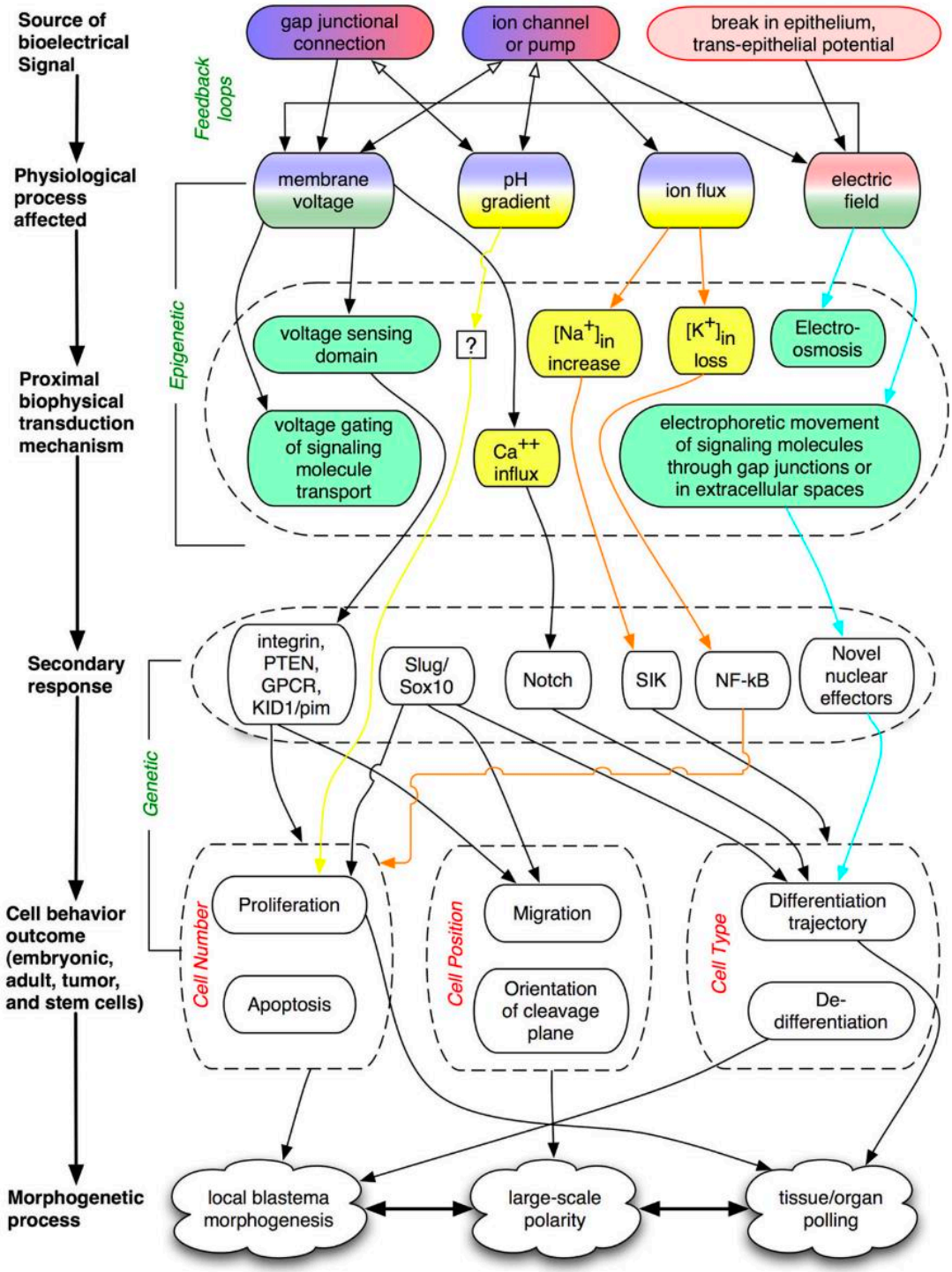

Figure 8: The bioelectric signaling, taken from Levin et.al. (74)

6 MIMICKING ENDOGENOUS ELECTRIC FIELDS TO TREAT CARDAIC REMODELLING

In the previous chapters, the researchers have demonstrated that endogenous EFs have a huge impact on the regenerative capacity of cells, bridging and guiding the chemical and genetic repair cascade and influencing cellular behaviors on a multi-stage scale. The exact molecular interactions that occur in specific organs, like the heart, are still unknown. Scientists are now trying to understand and map the complex patterns of effects between endogenous EF and the heart. There is a vast potential for EF involvement across a broad spectrum of cellular functions in the heart. Due to the diversity of the previously described effects of EF in the process of HF, mimicking endogenous EF might have a huge potential in treating heart failure and adverse cardiac remodeling.

6.1 Electrical stimulation and wound healing

The use of electrical stimulation has already reached direct application. Clinical acceptance of electrotherapy to induce wound healing is growing (76). It has been used to heal pressure ulcers in spinal cord injury (77), ischemic skin ulcers (78), to reduce visceral and abdominal fat in coronary artery disease (79), to reduce myofascial pain in the head, neck, and back (80–82), and to manage treatment sequelae in head-and-neck cancer patients after radiation (83).

The rationale for using electrical stimulation for wound healing is the proven evidence that the injured tissue produces a current, the current of injury, which promotes healing, as explained in the previous chapters. However, the current of injury gradually decreases, resulting in delayed or limited wound healing (44, 45).

6.2 Mimicking injury currents to activate anti-inflammatory pathways

Lujan et al. (2013) formulated the hypothesis that mimicking the endogenous EF, in their case, the injury potential that arises after post-infarct cardiac remodeling, would activate anti-inflammatory pathways to improve post-MI outcomes. They hypothesized that external application of an electric field mimicking the current of injury is expected to accelerate the healing process by placing electrodes directly on the heart. This could mimic the natural endogenic electric field created following injury (injury current) and improve post-infarct cardiac remodeling.

Their focus was to reduce the sustained inflammatory reaction post-infarction and to use the external application of currents to coordinate the inflammatory cascade to ensure the optimal temporal-spatial regulation of the inflammatory response. Specifically, reducing the prolonged inflammatory reaction leading to cardiac fibrosis and ventricular remodeling, which worsens systolic and diastolic function. (See Fig. 11) (84)

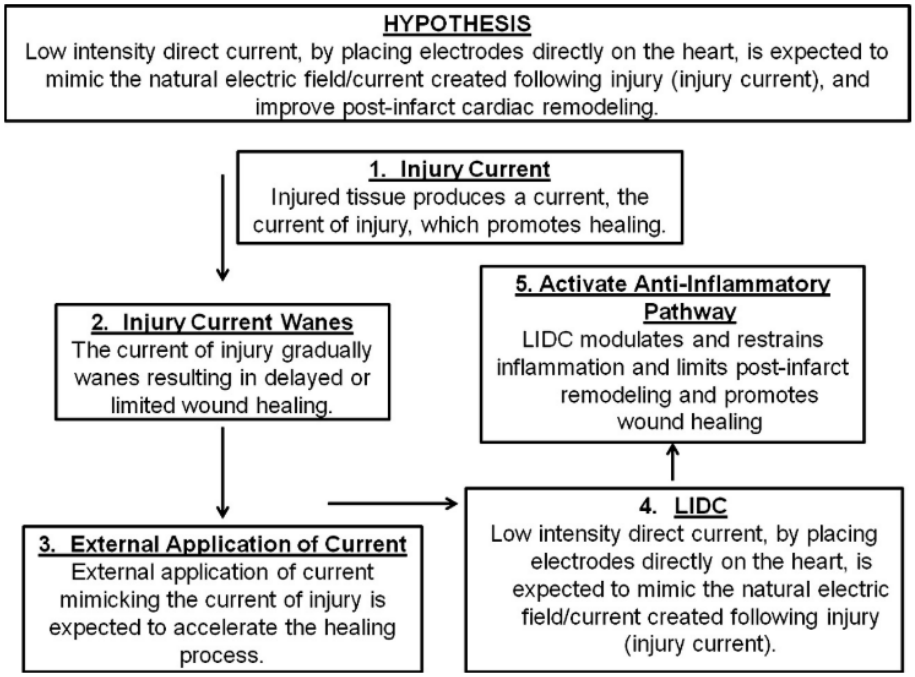

Figure 9: Hypothesis of mimicking endogenous electric fields to reduce cardiac remodeling in heart failure (taken from Lujan et. al.) (84)

6.3 Intracardiac devices used to treat heart failure

Device-based therapies have a long-proven success story in cardiology and cardiac surgery. Implantable cardiac defibrillators (ICD) for sudden life-threatening arrhythmias (85), as well as Cardiac Contractility Modulation (CCM) for delivering electrical signals to the failing myocardium during the absolute refractory period, have demonstrated their feasibility and safety (86, 87).

Device-based approaches to treating chronic heart failure have seen a major shift in the USA in recent years. Medicare and Medicaid services increased hospital reimbursement to increase access to new device-based technologies (88). To encourage faster and earlier innovative treatment strategies to cope with the chronic heart failure burden, the U.S. Food and Drug Administration (FDA) published new guidelines in 2013.

This step should encourage device-based companies to "address clinical needs and improve patient care, particularly when alternative treatments or assessments are unavailable, ineffective, or associated with a substantial risk" (89). The current landscape of device-related treatments is well summarized by the *Journal of the American College of Cardiology* (41). The designated devices are split out by heart failure subtypes (from HFrEF to EFpEF). Transcatheter edge-to-edge mitral valve repair (TEER), CCM, baroreflex activation therapy (BAT), and phrenic nerve stimulation are currently approved. The main device treatment groups are:

1. **Valve therapies:** MitraClip (Abbott), Tri-Clip (Abbott), Carillon mitral contour system (CMCS) (CARILLON), Medtronic's Intrepid transcatheter MV replacement (TMVR) system

2. **Autonomic modulation:** Splanchnic nerve stimulation, Vagus nerve stimulation, and baroreflex activation therapy

3. **Electrophysiological interventions:** Cardiac contractility modulation (OPTIMIZER System, Impulse Dynamics), and microcurrent therapy (C-MIC system, Berlin Heals).

4. **Respiratory modulation:** Asymptomatic diaphragmatic stimulation (VisONE), phrenic nerve stimulation for central sleep apnea

5. **Structural interventions**: Interarterial shunting, catheter-delivered basilar ventriculoplasty system (AcuCinch Guided Delivery system), left ventricular reconstruction

6. **Volume management**: Peritoneal direct sodium removal (Alfapump Direct Sodium Removal pump)

Device-based treatment approaches are generally the means of choice in disease progression and towards the end stage of cardiomyopathic disease (90, 91). Currently, there is only one device therapy in clinical trials that attempts to induce reverse remodeling of cardiomyocytes by using microcurrent (41). It is a new treatment possibility that uses permanent non-excitatory subthreshold microcurrent directly applied to the heart and completely independent of electrocardiogram status. It uses a coil electrode placed in the right ventricular cavity and a patch electrode, which is placed intrapericardially and directly on the epicardium of the left ventricle. The device set-up itself is very similar to regular implantable cardiac pacemaker.

The device is produced by the company Berlin Heals and is called the C-MIC device (cardiac microcurrent device). The system consists basically of four parts, of which three are implanted: a transvenous lead, an epicardial lead, and a power source to which the leads are connected. Both leads have the function of transmitting microcurrent to the heart (see Figure 13). The endocardial right ventricular coil is transvenously placed into the right heart and anchored. Its main function is to serve as a counter electrode. The PUT is needed for programming and reading out the data recorded by the device. The microcurrent parameters are set at implantation but can be modified via the PUT. Parameters and error messages are recorded and can be read out by the PUT. The PUT communicates wirelessly by using an adapter with the *C-MIC* device.

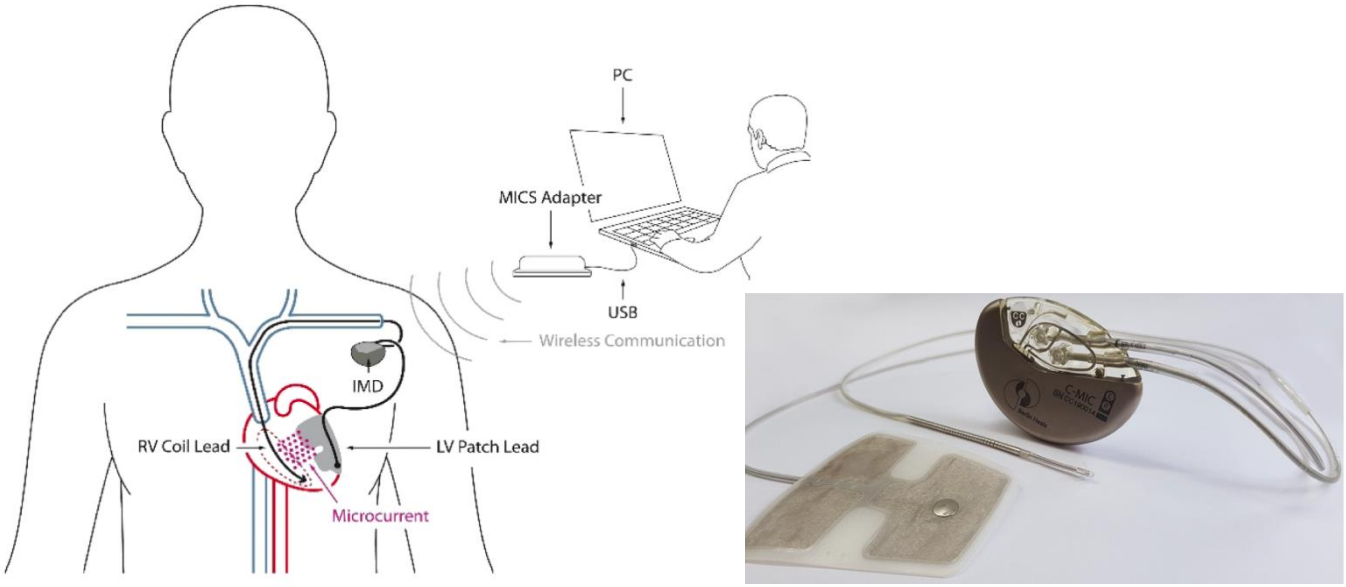

Figure 10: Schematic system configuration and picture of the actual device from Berlin Heals (IMD = implantable microcurrent device); (MICS = Medical Implant Communication Service) (92)

Its main clinical intention is to bridge the treatment gap for New York Heart Association (NYHA) Class III patients who have mortality rates of 26% during the 20 months after diagnosis and even 76% within 8 years (including treatment) (93, 94). The application of this microcurrent is based on the various cellular and extracellular effects that the reviewer will analyze in the following process. To conclude, the current of injury promotes the cellular healing process and guides cellular regeneration. The C-MIC device intends to mimic these currents and thereby try to modulate and guide the cellular regeneration process 92, 94). Nevertheless, clinical data which proves the effectivity of the C-MIC device is still sparse and will be evaluated in the further course of this thesis.

7 LITERATURE SEARCH STRATEGY

7.1 Aim

This review tries to identify the effects of exogenous subthreshold microcurrent stimulation of cardiomyocytes and/or progenitor cells or the whole heart and to investigate whether these effects can improve cardiac function in failing hearts.

7.2 Objectives

1. To identify various types and parameters of microcurrent/electrical stimulation used on cardiomyocytes, progenitor cells, and/or the heart in the scientific literature.
2. To investigate the effects of mimicking endogenous electric fields or bioelectric signals by passing an electrical microcurrent through cardiomyocytes, progenitor cells, and/or the heart.

7.3 Search Strategy and Screening

A search strategy was developed by the reviewer and was applied to Medline, Google Scholar, Epistemonikos, Scopus, and Cochrane Databases online databases until February 2022. In addition, the references of full-length manuscripts and review articles eligible from the initial search were reviewed manually to find other studies not captured otherwise (visualized in Appendix I). I screened titles and abstracts and the following full-text studies with the filters (see Appendix 1I, 95). Questions and unclear cases were resolved in consultation with the supervisor, who acted as a second reviewer. No automation tools were used in the selection process.

7.3.1 Literature search methods

The first step beginning my literature search strategy was to analyze the most important elements in the research question and to work out. Unfortunately, there is no scientific term that describes the use of non-excitatory subthreshold microcurrent stimulation. The reviewer used the guided methodology from Bramer et al. (96) to develop my search strategy. To identify a broad variety of elementary terms which describe the above-mentioned effect, the reviewer used the review by Pamela E. Houghton (97), using various types of electrical stimulation in clinical settings as a reference, as well as the previous mentioned large reviews regarding

endogenous electrical and electromagnetic stimulation (44, 45). The following two elements from the inherited thesaurus have been selected:

1. Microcurrency, Microcurrent, Electrical stimulation (ES), Electrical current (EC), Direct current (DC), Electric field (EF), Applied electric field, Endogenous electric current/field
2. Heart failure, Cardiac Failure

Each element chosen is then translated into keywords used by the above-mentioned online databases. These thesauruses functioned as the starting point for collecting potentially relevant search terms to formulate the search strategy. Additionally, the reviewer used truncations (most databases use the asterisk * for that purpose) to identify variations of words with the same word stem. The collected terms are then combined into a search strategy using parentheses and the Boolean operators OR between synonyms and AND between elements. This method can greatly increase the retrieval of relevant references. The search used field codes appropriate for the databases and limited the searching process of non-thesaurus terms to title and/or abstract, and author keywords (98). The finalized search strategy can be seen in Appendix 1I.

7.4 Selection process

7.4.1 Inclusion and Exclusion criteria

The inclusion criteria were set to collect a broad range of studies that used various methods of electrical stimulation (microcurrent) and narrow down my field of interest with the subsequent data selection process. The exclusion criteria should remove previous heart failure treatments that have already been established like CCM or CRT from the selection process, as well as any kind of above-threshold stimulation that cannot be defined as microcurrent. As this field of research is still new and mostly unknown, all outcomes of interest were included, as well as any setting in which the study was taking place (seen in table 1).

7.4.2 Selection procedure PRISMA

The following selection process was visualized by the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart (99). In total, 1435 papers have been identified using the developed literature search strategy seen in Appendix 1. Based on the information in the titles and abstracts, we were able to exclude 1125 articles. 48 full articles have been fully reviewed, and 5 have been excluded because of duplication.

Eligibility criteria for studies in the review			
		Inclusion criteria	**Exclusion criteria**
i.)	Population and conditions of interest	• Studies with cardiomyocytes, cardio progenitor cells, stem cells, endothelial cells • Heart failure hearts • Pilot studies	• Non-cardiac tissue • Non-cardiac failure hearts
ii.)	Study type/designs	• Human/Animal studies • Cell culture studies • All kind of study types • English literature • Full text articles and abstracts	• Non-english literature • Published before 2000 • Grey literature
iii.)	Interventions or exposures	• Continues subthreshold electrical stimulation of any kind on cardiomyocytes or the whole heart • Microcurrent	• Magnetic stimulation • Cardiac contractility modulation (CCM), cardiac resynchronization therapy (CRT) • High frequency pacing • Defibrillation of any kind • Above-threshold stimulation
iv.)	Outcomes of interest	• Molecular changes in the heart • Structural changes in the heart • Systematic physiological changes in the human organism	• N/A
v.)	Setting	• Any setting	• N/A

Table 1: Eligibility criteria

An additional 9 articles have been excluded because they do not match the research topic. 41 full articles were assessed for eligibility. Of these 41 articles, 4 were not of therapeutic interest, 6 used electrical stimulation for therapeutic purposes, 3 have not provided any data, and 5 have not been related to the heart. The 21 extracted articles and abstracts were grouped into three categories: preclinical in vitro, preclinical in vivo translational studies, and clinical trials.

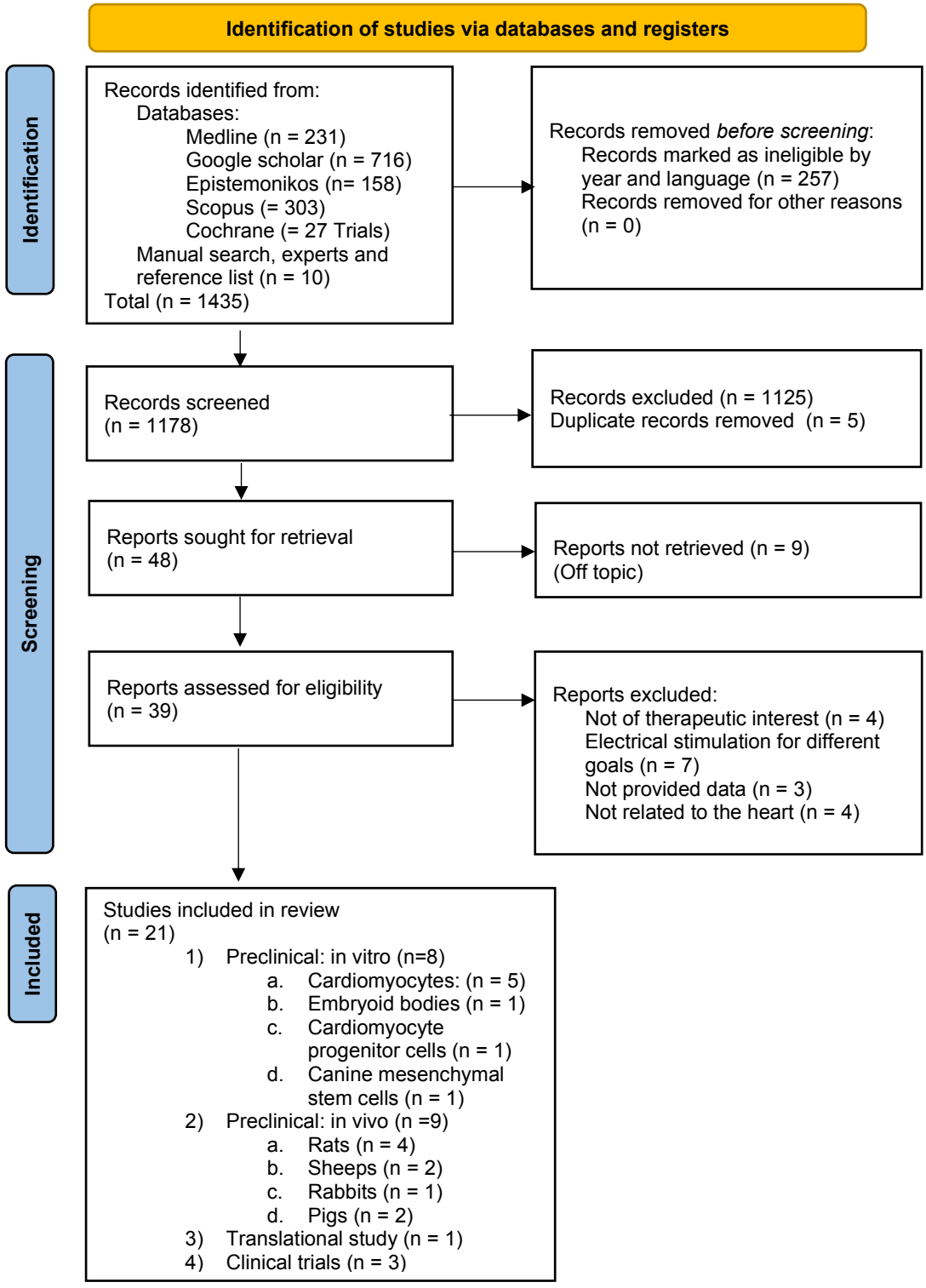

Figure 11: PRISMA flow chart 2020 (99)

8 DATA EXTRACTION

8.1 Methodological approach

After extracting and grouping the articles as shown in Figure 15, all chosen articles have been screened using the PICO (patient/population, intervention, comparison, outcome) framework to identify key terms to use in a search for evidence of the research objectives (100). Since many different study designs were used, the reviewer decided to extract the data according to the following criteria using an Excel table (see Appendix 2). The reviewer will then independently extract the data and reach a consensus together with his supervisors.

8.1.1 Data extraction criteria

a) Author
b) Year
c) Study type
d) Type of tissue/organ
e) Type of non-excitatory subthreshold microcurrent stimulation
f) Objective of the study
g) Primary and secondary endpoints
h) Methods used
i) Conclusion

8.1.2 Data analysis

Outcome criteria which have been analyzed:

1) Type of device used:
    a. C-MIC vs. different device
    b. Strength of the non-excitatory subthreshold microcurrent stimulation used (Volts per millimeter (V/mm), micro amperage (µA), hertz (Hz))
2) Preclinical – in vitro + in vivo studies
    a. Effects noticed
3) Clinical trials – case reports and first phase I trial
    a. Clinical data

Full text blocks were retrieved from the selected studies according to the data extraction criteria. In the next step, the outcome criteria will be analyzed according to the information retrieved from the Excel table, and the various effects of the different non-excitatory subthreshold microcurrent stimulations will be analyzed and synthesized qualitatively. Some results will be visualized in tables. Furthermore, the extracted data will be divided into preclinical (in vivo and in vitro results) and clinical case reports, translational studies, as well as clinical first-phase I trials.

9 RESULTS

We presented the results as a qualitative synthesis due to substantial differences among the studies concerning the methodology of investigations and the type, time, object, and strength of microcurrent used.

9.1 Parameters of electrical stimulation: summarized in tables

As outlined in Figure 15, the reviewer screened 21 full articles and abstracts. In 5 studies the C-MIC device from Berlin Heals was used with various intensities. In one study, from J. Rame et. al. it is most likely that the C-MIC device was used, as the study was supported by the Berlin Heals company, but no clear remark was made. The other 15 studies used individual customized approaches (see table 2).

The studies which have used the C-MIC device from Berlin Heals (92, 94) have chosen direct current stimulation of 0.35 μA to a maximum of 3072 μA and a duration ranging from 2h. 14 min. to a maximum of 195 days of stimulation. Not all studies reported the type and intensity of the microcurrent stimulation used. All other studies which have used the C-MIC device and other customized approaches but have not reported their intensity and time of microcurrent are listed in the appendix. All extracted results are represented in the table below.

Studies	Time	Microcurrent	Additional parameter
Mueller, Johannes et. al. (2005)(106)	7 days	DC (0, 20, 40, 60, 80, 100 µA)	cell culture conditions (+37°C, 5% CO_2)
Mueller, Johannes et. al.(2006)(107)	90 h	DC, 50 µA and 100 µA	
Mueller, Johannes et. al.(2006)(108)	90 h	DC, 50 µA and 100 µA	
Macfelda et. al. (2015)(110)	72 h	DC, 10 µA and 100 µA	
Kapeller B et. al. (2016)(112)	7.7 ± 0.9 h per d.	DC ~1 µA	Period 24.3 ± 6.1 d.
Macfelda et. al. (2017)(111)	8.5 h per d.	DC 0.35 µA	Period of 45 ± 3 d.
Mueller, Johannes et. al.(2017)(109)	Continuous for the whole period	DC 180 µA (~ 5 µA per cm^2)	Period of 195 d., active surface area: 36 cm^2
J. Rame (2021)(113)	Increasing - decreasing	DC, 0 - 3072 µA	For 2 h. and 14 min.
Elena Seerena et. al. (101)	1 or 90 sec.	DC, 1 V/mm, single electrical field pulse	Freely suspended for 3d. and then transferred to adhesion culture until day 19
Llucià-Valldeperas et al. (102)	Continuous	AC, 2 ms monophasic squarewave pulses of 25 mV/cm at 1 Hz	For 7 d. and 14 d.
P. Zhang et. al. (103)	30 min per d.	DC, 4.0 V/cm	For 4 weeks
L. Wen et. al. (104)	1, 3, and 6 h. per d.	AC, rectangular, 2 ms, 2 Hz and 40 µA	For 4d.
R.Mukherjee et. al. (105)	Continuous	AC, 5 ms, 2 µA, 4 V/cm pulses, 4 Hz	For 24h.

Table 2: Type of microcurrent used for electrical stimulation - micro amperage (µA), hertz (Hz), alternating current (AC), direct current (DC), carbon dioxide (CO2), day (d.), hours (h.)

9.2 Preclinical – in vitro + in vivo studies: summarized in tables

The literature search revealed 8 *in vitro* studies that used customized microcurrent stimulation systems to stimulate cardiac cell lines. Additionally, in all 9 *in vivo* studies analyzed, 6 study groups used customized microcurrent stimulation systems and 2 research groups used the C-MIC device to stimulate animal cardiac tissue. The C-MIC device has been used exclusively for *in vivo* studies and clinical trials. No customized system has been used for clinical trials. All *in vitro* and *in vivo* results have been extracted in tables and are represented in the appendix.

9.3 Clinical trials – case reports, translational studies and first phase I trial

9.3.1 Results: Translation studies

J. Rame et. al. (113) reviewed whether the direct application of an external electric current (in the physiological µA range) together with an electrical field projected onto the failing myocardium can explain the functional improvement of the hearts by edema reduction triggered by electroosmosis. There is a high probability that microcurrent might normalize the electrical environment with the consequence of increasing lymphatic flow by supporting the electrokinetically induced transport of electroosmosis and may also influence the charge of the glycocalyx. Using a sheep model for reference, they demonstrated the time correlation of microcurrent application and cardiac output. An increase in microcurrent is paralleled by an increase in cardiac output (visualized in figure 12).

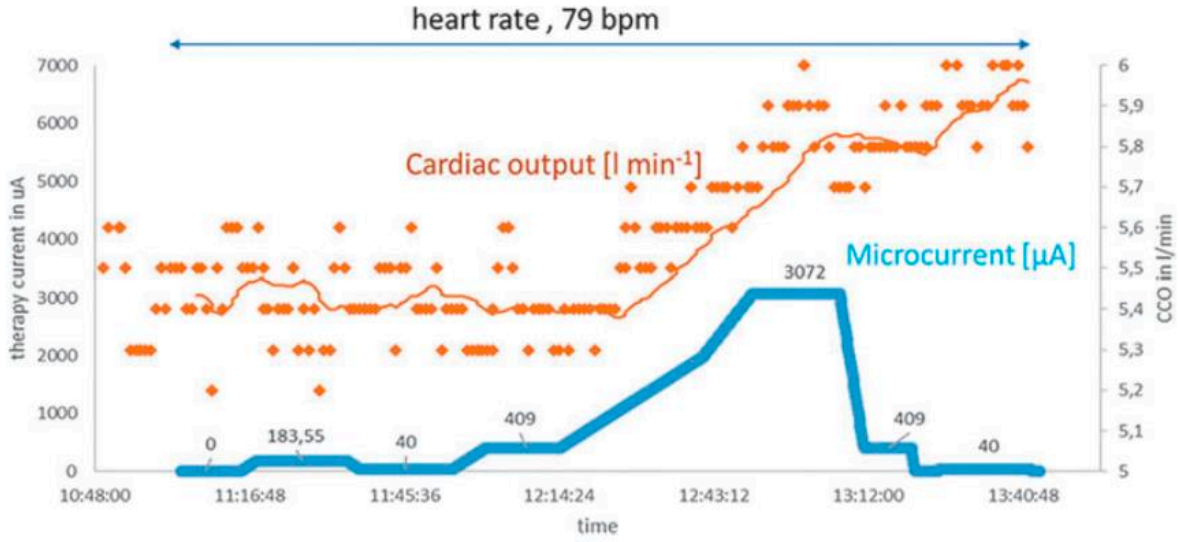

Figure 12: Proportionality of microcurrent application and cardiac output (113)

9.3.2 Results: case report

My literature search identified 1 case report from Schmitto et al. (94), who reported the first-in-man results with the C-MIC device from Berlin Heals. A 79-year-old patient suffering from HFrEF (dilated cardiomyopathy, NYHA class III, left ventricular ejection fraction 30%), with preserved right ventricle (RV) function and competent aortic bioprosthesis, successfully underwent implantation of the C-MIC device through left anterolateral thoracotomy. The interdisciplinary heart team judged that the patient was not suitable for alternative device therapies like CRT-D, mechanical circulatory support, or heart transplantation surgery. The intraoperative and postoperative course was uneventful, with regular mobilization and stable clinical parameters. The patient's clinical status was monitored in-hospital for the following 10 days.

At 30-day follow-up, the C-MIC device continuously operated without malfunction, and no electrical interference between devices was observed during hospitalization and at 30-day follow-up (see figure 18). The LVEF increased during the 30d. from 30% preimplant to 40% postimplant. Adverse effects on the cardiovascular system as well as on other organ functions were not observed.

Table 1. Patient's Vital Signs, Laboratory, and Echocardiography Data

Variables	Preimplant	Discharge	30 days
Vital signs			
Heart rate, beats/min	57	80	55
Systolic blood pressure, mm Hg	141	140	113
Diastolic blood pressure, mm Hg	53	83	53
Echocardiogram			
LVEF, %	30	35	40
LVEDD, mm	6.63	6.45	6.6
LVESD, mm	5.37	5.56	5.34
PAPs, mm Hg	32		28
Laboratory data			
White blood cells, x1,000/mL	13.5	10.6	9.0
Hemoglobin, g/dL	12.6	11.3	9.9
Platelets, x1,000/mL	176	179	230
AST, U/L	29	n.a.	21
ALT, U/L	31	n.a.	12
Creatinine, µmol/L	134	175	142
CK, U/L	24	37	31
NT-proBNP, pg/mL	4,805	n.a.	4,529

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; n.a., not available; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

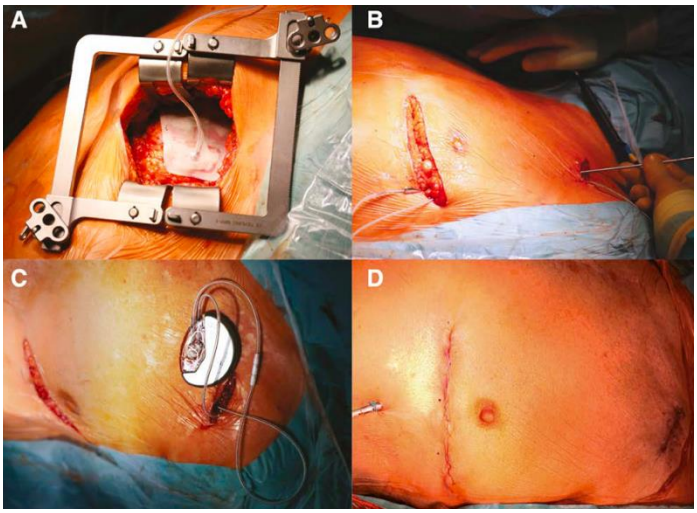

Figure 13: Clinical results taken from Schmitto et. al, intraoperative view: implantation through left thoracotomy, using minimal-invasive surgical technique, LV patch lead was positioned on the LV epicardial surface and fixated, both LV and RV leads were connected to the C-MIC generator (A-D) (94)

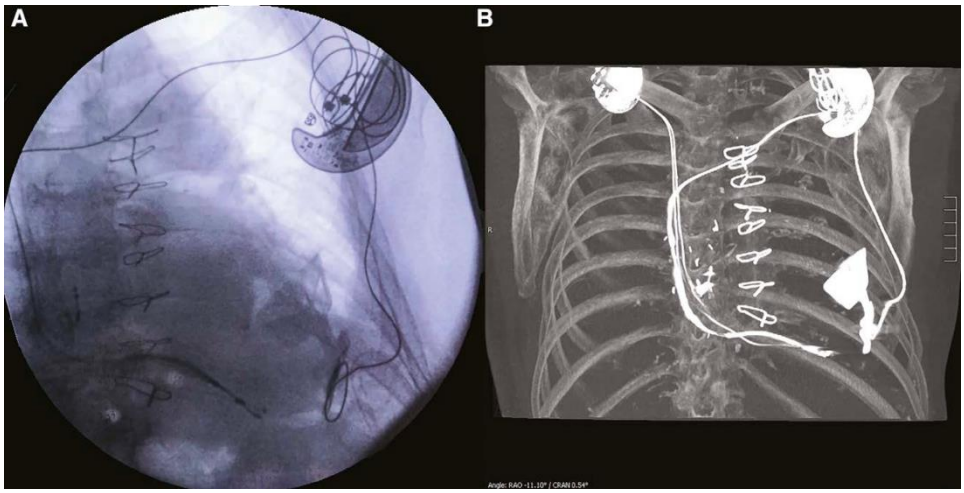

Figure 14: Angiographic view (A) and postoperative computed tomography scan (B) (94)

9.3.3 Results: pilot studies

The literature search revealed one single-arm, non-randomized pilot study with 10 patients (9 men; mean age, 62 12 years) at two sites in Europe with a 6-month follow-up to investigate whether the C-MIC device from Berlin Heals is safe, feasible, and improves cardiac function. The findings of Kosevic et al. (92), should pave the way for a future two-arm, randomized, controlled Phase II trial. The participants of the study included NYHA Class III heart failure patients and non-ischemic dilated cardiomyopathy with a left ventricular ejection fraction (LVEF) of 35%, treated with optimal pharmacotherapy. Specific endpoints are as follows: feasibility and safety in terms of the incidence of adverse reactions. Signs of efficacy were all-cause mortality, improvement of cardiac performance demonstrated by LVEF, LV end-diastolic diameter (LVEDd), and LV end-systolic diameter (LVEDs) (noted by quantitative echocardiography), the 6-minute walk test, NYHA classification, and the health-related quality of life using the 36-Item Short-Form Health Survey (SF-36) questionnaire. The controls and measurements started on day 10 after implantation, and at 2 and 4 weeks, and at 2, 4, and 6 months after implantation.

Application of the C-MIC device showed improvements in LVEF, LVEDd, LVEDs, and distance walked. After 14 days of microcurrent application, the average LVEF of patients with NYHA Class III heart failure improved by 8% points, and the mean 6 minute walking distance increased by nearly 100%. In addition, mean LVEDd decreased significantly (> 7 mm). Eight patients improved from NYHA Class III to Class I (most of them 14 days post-operatively); one patient improved to Class II; and another one to Class II/III (seen in figure 18). Additionally, in 9 patients, mitral valve regurgitation improved.

The conclusion of the first clinical trial applying microcurrent directly to the heart is that it is feasible and safe and can be used regardless of electrocardiogram findings.

Table 2 Baseline and follow-up clinical characteristics

Variable	Baseline	14 days		6 months	
	N = 10	*N* = 10	*P* value	*N* = 10	*P* value
Systolic blood pressure (mmHg)	117.4 ± 15.2	109.3 ± 8.6	0.128	124.1 ± 15.2	0.114
Diastolic blood pressure (mmHg)	73.3 ± 6.8	67.9 ± 7.4	0.201	73.5 ± 9.7	0.956
QRS complex duration (ms)	98.9 ± 14; r, 86–134	96.2 ± 9.9	0.902	104.1 ± 11.9; r, 96–134	0.078
LVEF (%)	31.8 ± 3.9; r, 26–35	39.8 ± 6.9	0.001	41.9 ± 9.0; r, 29–54	0.005
LVEDd (mm)	63.9 ± 3.2; r, 60–68	56.4 ± 3.3	<0.001	57.6 ± 4.9; r, 53–68	0.005
LVEDs (mm)	50.9 ± 7.0; r, 40–59	43.3 ± 5.1	0.007	44.4 ± 7.0; r, 37–62	0.002
6 min walk distance (m)	210.3 ± 38.5; r, 149–270	404.3 ± 49.0	<0.001	418.5 ± 47.4; 300–493	<0.001
NYHA class, no. of patients (%)	III, 10 (100)	I, 7 (70) I/II, 1 (10) II, 1 (10) III, 1 (10)	0.004	I, 8 (80) II, 1 (10) II/III, 1 (10)	0.002
SF-36 total score					
PCS	41.0 ± 4.1	53.1 ± 3.4	<0.001	57.2 ± 2.9	<0.001
MCS	31.6 ± 8.6	57.7 ± 4.8	<0.001	59.3 ± 4.8	<0.001

LVEDd, left ventricular end-diastolic diameter; LVEDs, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; MCS, Mental Component Summary; NYHA, New York Heart Association; PCS, Physical Component Summary; r, range; SF-36, 36-Item Short-Form Health Survey questionnaire.
Values are presented as mean ± SD.

Figure 15: Results from the first single-arm, non-randomized pilot study, from Kosevic (92)

Mueller et al. (119) published the results of eight patients (7 men, 1 woman; mean age 56.3 ±8.5 years, history of HF 2.9± 0.7 years) who received the C-MIC system for the application of microcurrent to see if it is safe and practicable and improves cardiac function. His findings backed up those of Kosevic et al. LVEF improved from 30.9 ±4.2 % at baseline to 38.1 ±3.2 %, 42.5±5.6 %, and 43.3±4.4 % after 3 days, 10 days, and 1 month, respectively, and maintained the improvement throughout time. The LVEDd was reduced from 63.6±3mm (baseline) to 59.6±3.8mm (after 3 days), 58.4±3.6mm (after 10 days), and 55±2.9mm (after 2 months) on echocardiography. The results of the 6-minute walk test increased from 186.8±37.2m (baseline) to 382.3±42.5m (after 10 days) and 416.7m (after 1 month) and remained stable in the further course of the study. All patients started in NYHA class III before microcurrent application and improved to NYHA class I after 1 month, where they remained thereafter. Moreover, on electrocardiogram (ECG), one patient with a left bundle branch block (LBBB) and a QRS complex width of 130 milliseconds (ms) at study start improved to 92 ms after 10 days of C-MIC treatment. Mueller et al. confirmed the safety, feasibility, and quick tempo of cardiac functionality after microcurrent application.

10 DISCUSSION

To my knowledge, this is the first systematic literature review that has explored the relationship between endogenous EFs that arise during processes of cellular regeneration of injured cells and mimicking those by using externally applied electrical stimulation on cardiomyocytes with various intensities to improve impaired cardiac function.

First, the researchers described the burden and need for effective and individual treatment approaches, especially for chronic heart failure patients, who become difficult to treat and still progress at the end stage of disease despite using optimal pharmacotherapy. Next, the pathophysiological mechanisms of cardiac remodeling, including cardiac fibrosis, inflammation, edema, and hypertrophy has been reviewed. Moreover, existing therapeutic approaches that might inhibit these processes have been analyzed.

It has become evident that the complex interactions during cardiac remodeling cannot be tackled using single-factor approaches. Second, we introduced the principle of endogenous EFs and their effects on cell biological processes like regeneration and cell migration (proliferation and apoptosis), as well as immune cell modulation and many more. Furthermore, we linked those endogenous effects to the possible interactions that arise during cardiac remodeling. Third, we presented the current usage of electric stimulation in clinical practice and presented the hypothesis that mimicking endogenous EFs might be a promising modality to interrupt the vicious cycle of cardiac remodeling. Fourth, we introduced the current established device-related therapies in the United States, and we presented the C-MIC device from Berlin Heals, which is currently mentioned in scientific literature as the only device in clinical trials that uses microcurrent application to induce cardiac reverse remodeling. Following this complex thematic introduction, we conducted a systematic literature review with the aim of identifying types and parameters of microcurrent application as well as possible effects that follow direct application on cardiomyocytes, progenitor cells, or the heart.

Now, we will analyze the different types and intensities of electrical subthreshold stimulation used in the previously mentioned studies. The type and intensity of electrical stimulation used to achieve the designated effects are mostly unknown and were not reported as those. The two main groups of electrical stimulation used in my literature research have been DC (10 studies) and AC (3 studies). The other eight remaining studies have not published their electrical stimulation protocols.

Direct current, also known as galvanic current, describes the flow of charged particles in one direction (unidirectional) with low amperage and low frequency (μA to 1 μA, 0.5–100 Hz) and is therefore, by definition, below threshold stimulation (97, 120). As previously stated, (44, 121, 122), DCs play important physiological roles in regeneration and development.

Biphasic current (AC), is the flow of charged particles in which a change in direction occurs at a minimum once every second and where dipole rotations oscillate. It is not regarded as physiologic stimulation (49, 97). The applied electrodes continuously alternate their polarity each cycle and stimulation is used for example in transcranial alternating current stimulation (tACS) to modulate cognitive processes, but is not used to modulate cellular regeneration processes or in order to mimic endogenous EFs (123).

Studies which investigate the endogenous and exogenous effects of AC current application are more controversial and produce inconsistent outcomes (49, 124). It was interesting to note that all three studies that used AC stimulation used low amperage (50 μA) and low frequency (5 Hz) electrical stimulation, both of which are considered below threshold electrical stimulation.

DC application follows the principle of mimicking endogenous EFs and uses the endogenous mechanisms of bioelectricity as stated previously. Nevertheless, there is no clear evidence in the literature about the differences between DC and AC used for electrical stimulation on cardiomyocytes or the whole heart, nor can advantages or disadvantages be evaluated as most of the data published is based on empiric evidence.

To evaluate the intensity of μA used in DC studies, one must know the endogenous DC electric field strength, time, and distribution for cardiomyocytes in mV/mm, which drives the regenerative process. It is most likely the case that different magnitudes of the applied electric field initiate different cellular response mechanisms at different organizational levels, involving the whole cellular gamut of regeneration (74, figure 9 (74), 44). Today, we know that regenerative currents in newt limbs of between 10 and 100 μA/cm2 create a steady voltage drop of 60 mV/mm within the first 125 μm of extracellular space, and this is described in literature as essential for regeneration and development (44, 125). Direct measurements have revealed that using bovine cornea and human skin, all cellular behaviors around 500 μm of the membrane injury are affected by the endogenous electric field (62). Therefore, it seems feasible that Zhang et. al. (103), Mueller et. al. (106–108), Macfelda et. al. (111), and Kapeller B et. al. (112) used DC of 100 μA intensity.

Future research must be dedicated to optimizing microcurrent stimulation protocols, providing more detailed information on DC and AC application modes as well as differentiating which intensity triggers the regenerative cascade most efficiently.

To summarize and analyze the *in vitro* effects using continuous DC microcurrent below threshold (106–108, 110, 112), it has shown that collagen types I and III, as well as MMPs and TIMPs, which are important mediators in cardiac fibrosis and cardiac remodeling, are highly influenced by microcurrent application. MMPs are proteolytic enzymes that are found in failing hearts, mediating the inflammatory signaling cascade by attracting inflammatory cells for necrotic removal as well as degrading the ECM, which results in a loss of structure and support of cellular units (126). These results also confirm the findings of Becker (1985) (45, 73), who formulated the thesis that collagen molecules are under the control of endogenous electric fields. Collagen type I is also associated with reduced ventricular compliance and increased myocardial stiffness (127, 128). Applying microcurrent has shown to influence cardiac remodeling by downregulating collagen I and III as well as MMPs. Furthermore, continuous DC microcurrent can reduce IL-6, which is an important proinflammatory marker in heart failure. Depending on the level and duration of its secretion and the presence of other cytokines, they can negatively influence cardiac contractility by modulating the ECM (129). IL-6 knock-out is associated with reduced cardiac fibrosis and weakened fibroblast response (130).

Additionally, DC microcurrent is able to increase molecular ATP production as well as ATPase efficiency in stimulated cardiomyocytes. During heart failure, the cardiac metabolism is significantly altered, which involves changes in substrate utilization and mitochondrial dysfunction, leading to reduced ATP generation, which is related to systolic dysfunction (131, 132). Applying external microcurrent might stabilize the cardiac energy metabolism of injured cardiomyocytes and support the energy deficit due decrease in mitochondrial oxidative capacity (133).

Furthermore, DC single electric field pulses for up to 90 sec. have been shown to regulate the process of cardiogenesis via the formation of ROS. This process is initiated by growth factors, hormones, or mechanical stress, which then triggers a small oxidative wave and produces ROS in low concentrations (134). Also, during MI and reperfusion injury, ROS will be generated because it is part of the signaling and activation of intrinsic myocardial repair capabilities (135).

ROS seems to take part in the regulation of cell proliferation, DNA damage, and cell aging (101). Applied microcurrent might influence second messengers downstream of the regeneration cascade and support the process of carcinogenesis.

Summarizing and analyzing the *in vitro* effects using AC microcurrent below threshold (102, 104, 105), it was shown that AC current supports the differentiation of CMPCs into a cardiomyocyte-like phenotype, enhancing cardiac gene expression. Cardiac progenitor cells might be electrically stimulated and used for cell transfer therapy, which could allow a selective replacement of important functional cell units like pacemaker cells or ventricular cardiomyocytes in the future (136). Additionally, AC stimulates S100A4 (104), a protein with significant angiogenesis effects as well as growth and survival-supporting effects in cardiomyocytes, especially occurring at the border zone after a myocardial infarction (137, 138). It also promotes cardiac differentiation of embryonic stem cell-derived embryoid bodies (139). Further studies must reveal the connection between the cardiomyocyte necrosis border and possible applied AC electrical fields that might upregulate S100A4.

Results from R. Mukherjee et al. have shown that AC increased regional stiffness in the MI region of mature pigs, reducing certain MMP subtypes and increasing TIMP-1 levels, which therefore prevents myocardial thinning post MI. A similar result was seen with stimulated fibroblasts that were extracted from the porcine model of MI. AC was also found to induce a TGF-ß signaling pathway and promote fibrillar collagen secretion from fibroblasts or myofibroblasts (140). Future studies must evaluate the risk of increased fibrotic response due to increased TGF-ß, which comes at costs of preventing myocardial thinning after MI.

The effects of AC applied microcurrent have shown a potential benefit of inducing angiogenesis and increasing the ECM via TGF-ß despite being classified as non-physiological current. Future research is needed to evaluate the mechanism of action and signaling pathway of AC currents.

Analyzing the *in vivo* results (103, 105, 109, 111, 114 - 118) of microcurrent application showed interesting results. Only DC electrical stimulation was used. Additionally, the C-MIC device was used in two animal models, which uses DC currents as well. It seemed plausible that the researchers had the intention of mimicking physiological current intensities to initiate and support regenerative effects in heart failure models.

The first pilot study (109) applying continuous application of microcurrent to the failing hearts of SHR had remarkable results. They have reported statistically significant changes in gene expression of ECM components, including collagen type I, TIMP3, Cx43, and Cx45, as well as a reduction in IL-6 and TGF-ß. These findings support the results of the in vitro results using DC microcurrent application. Downregulation of TGF-ß, which in turn alters the secretion of collagen type I (as reported), might reduce cardiac fibrosis (4). Also, Mueller et al. (114, 115) reported a significant downregulation of IL-6 and TGF-ß in their in vivo SHR study group, supporting the anti-inflammatory effects of microcurrent application.

Moreover, it has been established that controlling chronic inflammatory processes is a key working point to limit cardiac remodeling (2). Today we have evidence that macrophages, one of the important cells in inflammation and regeneration processes, are directly influenced by aEFs using physiological ranges. The phagocytic uptake of a variety of targets is enhanced, including carboxylate beads and apoptotic neutrophils. These changes are partly induced by clustering of phagocytic receptors, PI3K and ERK activation, mobilization of intracellular calcium, and actin polarization. Furthermore, cytokine secretion from macrophages is modulated by EFs in response to inflammation and healing (141).

Christina E. Arnold et al. (142) discovered that T-cells are also influenced by EFs of physiological strength. EFs strikingly downregulate T cell activation via reduced IL-2 secretion, influence T-cell migration towards the wounded site and proliferation on site, cytokine output and STAT3 signaling pathways regulating functional intracellular processes.Their results show a significant downregulation of T-helper cells (Th1, Th17) and T-regulatory cells (Treg) associated cytokines, interferon gamma (IFN), IL-17, and IL-10. Additionally, not only T-cells but also monocytes and B-cells are influenced by EFs and migrate towards the cathode. The activation of the lymphocyte migrating process shares similar features with the chemoattractant receptor signaling, suggesting that electrotaxis and chemotaxis use common intracellular cell motility programs (143). These outcomes show novel mechanisms by which EFs may influence T-lymphocyte behavior and provide a more complex understanding of how EFs influence wound healing, inflammation, and regeneration. Further research is needed to evaluate this behavior of lymphocytes in heart failure models using cardiac microcurrent application.

Zhang et al. (103) reported increased capillary density after DC microcurrent application. Electrical microcurrent stimulation has shown effects on the induction of angiogenesis, which incorporates a critical role in myocardial hypertrophy during heart failure.

In vivo experiments have shown that applied electric fields of small physiological magnitude, similar to endogenous EFs, directly stimulate VEGF production of endothelial cells in culture without the presence of any other cell type. Endothelial cells in culture were reoriented, elongated, and migrated in response to EFs as low as 75-100 mV/mm^{-1} (1.5-2.0 mV across an endothelial cell). Additionally, applied EF resembles the response of endothelial cells to fluid shear stress. These pre-angiogenic responses required VEGF receptor activation and were mediated through PI3K-Akt and Rho-ROCK signaling pathways, resulting in reorganization of the actin cytoskeleton. The hypothesized mechanism involves the secretion of fibroblast growth factor 2 (FGF2), which then activates the mitogen activated protein kinase/extracellular-signal-regulated kinase (MAPK/ERK) pathway, interacting with the fibroblast growth factor receptor (FGFR), which induces the growth of human microvascular endothelial cells (HMEC) (144). Applying electric DC to human umbilical vein endothelial cells (HUVEC) *in vitro* also resulted in activation of VEGFR2, Akt, extracellular regulated kinase 1,2 (Erk1/2), as well as the c-Jun NH2-terminal kinase (JNK). EFs not only induce the early events of angiogenesis from ECs but also have an integrative role during sprouting tubular formation. The highest growth rate was observed at 150 mV/mm (145). Also, Wei et al. (146) applied a DC electric field with a strength of 150 mV/mm to HUVECs. EF stimulation resulted in endothelial nitric oxide (eNOS) activation and nitric oxide (NO) production via a PI3K/Akt-dependent pathway. Additionally, endothelial cell proliferation was increased with an enhanced S phase during the cell cycle. The activation of eNOS seems to be an important key signaling pathway necessary for EF-mediated angiogenesis.

To summarize the effects, applied DC EFs influence endothelial cell migration and proliferation for wound healing, which in turn adjudicates capillary formation induced by electrical stimulation, a major role during regenerative cellular processes. Future research needs to confirm these promising findings and evaluate the neo-microvasculature formation in heart failure models induced by microcurrent application.

Analyzing the first *in vivo* clinical data (117) revealed significant normalization of the LVEF (up to 68% post microembolization, with an increase from 44.2 $\pm$13.4% to 62.4 $\pm$7.3%) within 12 weeks of microcurrent application. An increase was also noticed by Mueller et al. (109), although not statistically significant. No exact mechanism for this rapid increase in cardiac functionality *in vivo* has been suggested by research groups. It is interesting to note that a similar rapid recovery of LVEF has been reported with the C-MIC device in the first clinical phase I trial by Kosevic et. al. (92), Schmitto et. al. (94), and Mueller et. al. (119).

All report a rapid recovery of LVEF within weeks (up to 10% in 30 days (94)) as well as a reduced LVED (from 63.9±3.2 mm to 57±4.9mm (92)). Furthermore, two studies reported (92, 119) a significant improvement in NYHA class (from class III to II and I), as well as profound symptomatic improvement in evaluating the 6-min walk test. Analyzing these rapid effects of functional recovery, one cannot expect microcurrent to have induced reversed cardiac remodeling after a 2-week application period (147). Even following direct application of microcurrent *in vivo*, one could observe a parallel increase in cardiac output during the 2 hours of application. (114, see figure 17). This direct response suggests another explanatory mechanism with a direct application-effect causality.

One possible mechanism, supported by Kosevic et al. (92) and J. Rame (113) et al., which could explain this rapid improvement, is the direct impact of electrical guidance on the myocardial fluid balance. Taking the principle of electroosmosis, described as a "phenomenon of the movement of a liquid through a capillary vessel bearing a surface charge caused by an electrical field parallel to the surfaces of the vessel" (113), Today, there is evidence that electroosmosis is a consequence of endogenous EFs. Electrical fields that are applied to an electrolyte solution in the tangential direction of a charged surface influence the water transport on a micro or nanoscale. The backbone of the electrokinetic mechanism is the ionic polarized electrical double layer, which represents the bidirectional interface between solid and liquid media (148). Fischbarg and his colleagues showed that paracellular electro-osmosis of the corneal epithelium is a cyclical process, which is controlled by the drop and exhaustion of cellular Na^+ (149). Additionally, V. Andreev et al. developed two mathematical models which successfully demonstrate the importance of endogenous EFs in influencing the transport of negatively charged phosphorylated messenger proteins from the cellular membrane to the negatively charged nucleus, which would overcome the repellence forces which would normally appear on molecules of the same polarity (150). These implications provide a meaningful effect of electroosmosis on intracellular biological processes and might contribute to myocardial fluid homeostasis, which is highly disturbed during myocardial remodeling, as mentioned previously.

Thus, edema formation can be interpreted in part as a disturbed electrical environment that produces a potential gradient in the myocardium. This particular environment includes the negatively charged glycocalyx (intra-luminal) of the fluid exchange vessels, which controls the permeability of the capillary wall and, in turn, the fluid balance and pressure conditions in the interstitium.

Following this conclusion, an externally applied weak electric current together with an electrical field may stop the vicious circle of edema formation and reduced cardiac function. There is a high probability that microcurrent might normalize the electrical environment with the consequence of increasing lymphatic flow by supporting the electrokinetically induced transport of electroosmosis and may also influence the charge of the glycocalyx. A process which is seen in a sheep model, for reference, demonstrates the time correlation of microcurrent application and cardiac output. An increase in microcurrent is paralleled by an increase in cardiac output. Given the immediate stroke volume response, a mechanism that could explain the impact of microcurrent on the myocardium is a reduction in myocardial fluid content. Consistent with this observation is that a reduction in microcurrent does not lead to an immediate drop in continuous cardiac output because the process of fluid retention is subject to a physiological delay of reaccumulating fluids into the myocardium (113).

Rame et al. also argued that patients **without** myocardial edema will most likely not show a rapid increase in cardiac function immediately after the implantation, but that they might benefit from the anti-inflammatory effect of long-term application of microcurrent on myocardial function. They hypothesized that applying microcurrent directly to the heart would depend only on the extent of myocardial edema, which causes compromised heart function, rather than the overall heart failure. Using the "Helmholtz – Smoluchowski relation: $V_{EO} = \varepsilon\varepsilon_o\zeta V / \eta$ " (113) one can estimate the strength of the electrical field that must be applied to achieve a certain velocity of lymphatic drainage. Future clinical studies must prove this hypothesis made by J. Rame et. al. and evaluate the quantity of fluid removed using cardiac microcurrent.

Future studies must confirm the hypothesis of electroosmosis being the responsible regulator for the rapid functional recovery of cardiomyocytes, as well as establish clear evidence that myocardial microcurrent application is able to reverse the pathologies that arise during cardiac remodeling. *In vitro, in vivo* and the first clinical results show promising effects of microcurrent application and might evolve into a promising future treatment for heart failure patients.

All studies concluded that the use was safe and feasible. No direct adverse effects could be found. This could be due to various reasons. One reason could be the limited reports of microcurrent usage on cardiomyocyte tissue. Also, the intensity of microcurrent application uses low-intensity DC applications and or reduced-frequency AC applications, which produce no heating effects nor mechanical disturbances.

Additionally, applying the C-MIC device directly onto the human heart has not shown any adverse reactions. This could also be because the device has similar sizes and similar set-ups as the classical pacemaker defibrillator systems or the epicardial CRT leads, which are implanted on a routine basis (92).

There are several limitations to this review which need to be mentioned. Firstly, this is the first systematic review that analyzes the exogenous application of electrical stimulation on cardiomyocytes, and the author endeavored to produce a comprehensive and detailed analysis of this unknown research topic. The literature research revealed no accepted scientific terminology that describes the usage of subthreshold electrical stimulation used.
Many different terms like "electrical stimulation," "microcurrent," "low intensity direct current," and "exogenous applied electric fields" are used interchangeably by the different research groups from different countries. This diversity of terms increased the difficulty to find accurate inclusion and exclusion criteria of this research. Additionally, the quality of this review is highly dependent upon the quality of the included articles. The heterogeneity as well as the small number of studies included could be seen as limitations, but this was unavoidable considering the novelty of this application technique. Nevertheless, I tried to strengthen this review using a systematic literature search process and a detailed analysis about EF effects.

CONCLUSION

Preclinical *in vitro* and *in vivo* results together with the first published clinical trials have shown that below threshold microcurrent stimulation can exert a variety of cardiac-enhancing effects on cardiomyocytes and their progenitors. All studies confirmed different supportive bio-cellular effects ranging from inducing cardiac differentiation of progenitor cells, modulating the ECM components (MMPs, TIMPs, collagen, fibroblasts, connexins), altering the inflammatory signaling cascade, inducing angiogenesis, improving LVEF, influencing the cardiac metabolism, improving the cardio-regeneration capabilities of progenitor cells, and improving weaning and survival rates after RI. This novel treatment approach has shown that applying exogenous EF´s using DC or AC currents pre and post-injury modulates the regenerative processes, influencing a multi-scale downstream cascade of cellular effects which are all related to cellular coping mechanism during heart failure. Learning to control these dynamic bioelectric signals might enable new possibilities to tackle the burden of chronic and advanced heart failure syndromes and can represent a key step towards the development of innovative applications in regenerative medicine.

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12 APPENDIX

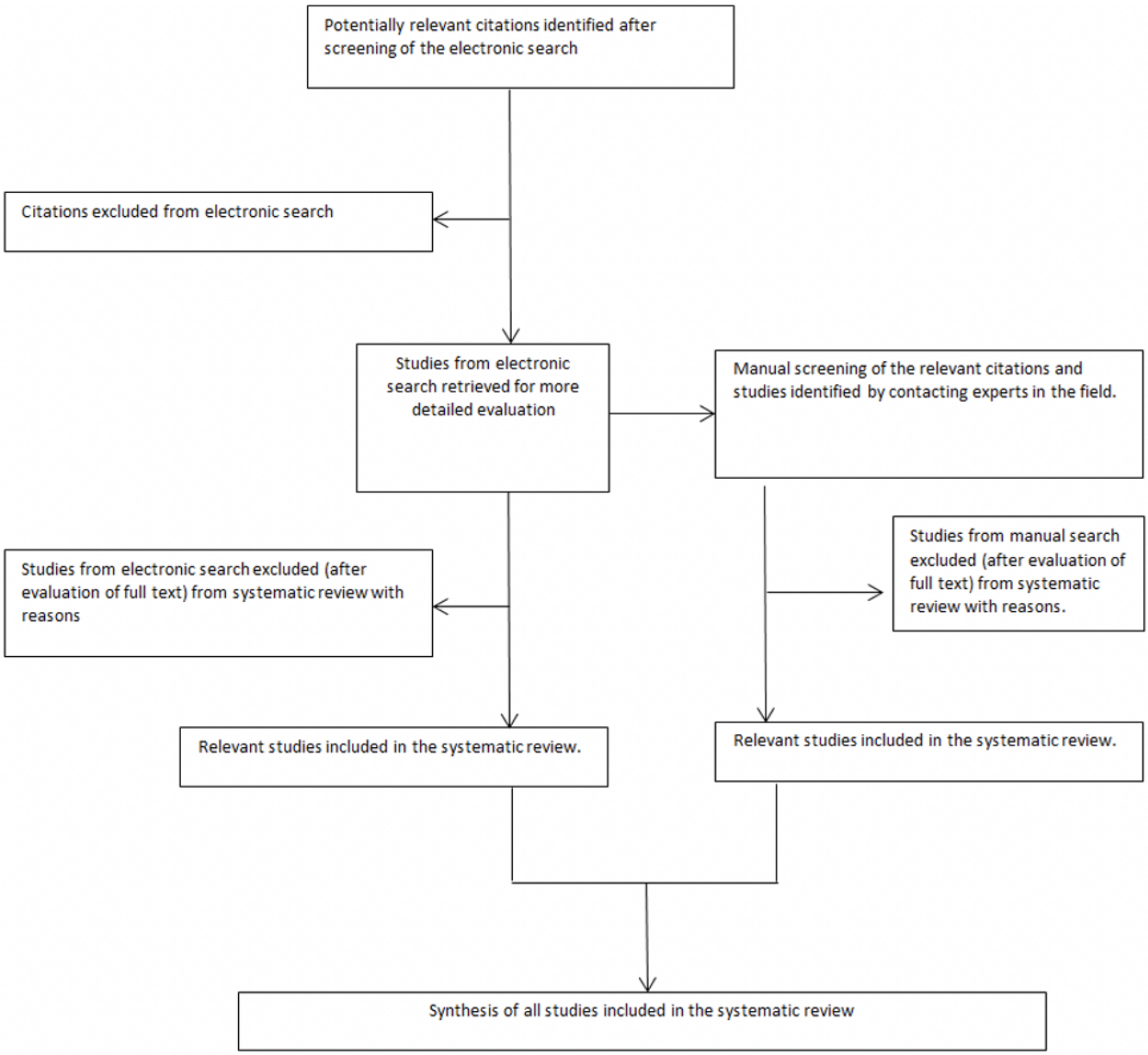

Figure: Taken from the static Springer-content platform (114)

Literature search strategy: A systematic review of the expected effectiveness of electric microcurrent in heart failure treatment

Concept 1: Microcurrent
“microcurrent*”[tw] OR “electrical stimulation” [tw] OR “electrical current” [tw] OR “direct current” [tw] OR “applied electric field*”[tw] OR “applied electric fields” [tw] OR “endogenous electric field*”[tw]

Concept 2: Heart failure:
"Heart Failure"[Mesh]

PubMed search:

#1	“microcurrent*”[tw] OR “electrical stimulation” [tw] OR “electrical current” [tw] OR “direct current” [tw] OR “applied electric field*”[tw] OR “applied electric fields” [tw] OR “endogenous electric field*”[tw]
#2	"Heart Failure"[Mesh]
#3	**#1 and #2 = n231**

Google scholar search:

"applied electric field" OR “microcurrent” AND "heart failure" OR "cardiac failure"
= n 716

Epistemonikos search:

(title:("microcurrent" OR "electrical stimulation" OR "electrical current" OR "direct current" OR "applied electric field" OR "applied electric fields" OR "endogenous electric field") OR abstract:("microcurrent" OR "electrical stimulation" OR "electrical current" OR "direct current" OR "applied electric field" OR "applied electric fields" OR "endogenous electric field")) AND (title:("heart" OR "cardiac") OR abstract:("heart" OR "cardiac"))
= n 158

Scopus:

TITLE-ABS-KEY (“endogenous electric field” OR “electrical current” OR “applied electric field” OR “microcurrent” AND “heart failure” OR “cardiac”)
= **n 303**

Cochrane:

"endogenous electric field" OR "direct current" OR "electrical current" OR "applied electric field" OR “microcurrent” AND "heart failure" OR "cardiac"
n = 27 Trials

TOTAL = 1435

Use of Micorcurrent for HF

Citation	Author	Year	Country	DOI	Reference	Study type	Type of tissue/organ	Objective of the study	Methods: details of the experiment/study	Results	Conclusions	Device	Type of non-excitatory subthreshold electrical stimulation
101	Elena Serenaa,b,1, Elisa Figallob,1, Nina Tandona,1, Christopher Cannizzaroc, Sharon Gerechtd, Nicola Elvassoreb,e,⁎, Gordana Vunjak-Novakovica,⁎	2009	USA/Italy	doi:10.1016/j.yexcr.2009.08.015.	Serena E, Figallo E, Tandon N, Cannizzaro C, Gerecht S, Elvassore N, et al. Electrical stimulation of human embryonic stem cells: Cardiac differentiation and the generation of reactive oxygen species. Exp Cell Res [Internet]. 2009;315(20):3611–9.	In vitro study	Embryoid bodies (EBs) derived from human embryonic stem cells	The effects of electrical field stimulation on ROS generation and cardiogenesis in embryoid bodies (EBs) derived from human embryonic stem cells, using a custom-built electrical stimulation bioreactor	Elena Seerena et. al. used a custom designed bioreactor filled with low ionic content buffer solution with a conductivity of 500 µS/cm for the electrical stimulation on ROS generation and cardiogenesis in embroid bodies. A single electrical field pulse of 1 V/mm and duration of either 1 or 90 seconds was applied to the embroid bodies. Cell viability after the electrical stimulation was verified 2 h after (single pulse and after 4 days of continuous stimulation - 1 V/mm for 5 ms, at 1 Hz)	1. ROS generation was significantly higher in electrically stimulated 4- and 8-day-old EBs than in the corresponding unstimulated Ebs 2. The expression of cardiac troponin T was observed in beating EBs. The sarcomeric organization of the cells, an ultrastructural marker of cardiac differentiation, was also verified 3. Application of electrical stimulation can enhance the number of cardiac-differentiated hESCs through mechanisms involving ROS generation	These results imply that electrical stimulation plays a role in cardiac differentiation of hESCs, through mechanisms associated with the intracellular generation of ROS	Customized	Electrical field pulse of 1 V/mm and duration of either 1 or 90 s.
106	Johannes Müller, Barbara Kapeller, Gerd Wallukat, Karin Macfelda Berlin Heart, Berlin, Germany Institute of Biomedical Research, Vienna, Austria	2005	Austria/Germany	doi:10.1016/j.eupc.2005.02.041	Mueller, Johannes & Kapeller, Barbara & Wallukat, Gerd & Macfelda, Karin. (2005). Changes of the Collagen composition in the heart caused by microcurrent application. An explanation for the improvement of cardiac function by bi-ventricular pacing?. EP Europace. 7. 298-298. 10.1016/j.eupc.2005.02.041.	In vitro study	Cardiomyocytes	To examine whether microcurrent can influence the collagen synthesis in the myocardium	Adult cardiomyocytes were isolated and cultivated in 24 well cell culture plates. Current of different magnitudes (0, 20, 40, 60, 80, 100 :A) was applied via platinum electrodes by a special custom-made device. The whole equipment was incubated under cell culture conditions (+37°C, 5% CO2) over a period of 7 days. Changes of the collagen type I and type III synthesis were analyzed using immunohistochemical staining methods. Collagen type I and type III content was quantified using a special fluorescence confocal laser scanning microscopy system including special analysis software	1. After exposure to a moderate current magnitude (40, 60 µA), cardiomyocyte collagen type I production did not change significantly. 2. When exposed to high current (80, 100 µA), he noted a highly significant mean drop of 20.6%. Collagen type III showed a mean rise of 29.7% when exposed to moderate current and a decrease of 25.2% when exposed to high current. 3. In addition, the cell growth rate was shown to be higher at moderate and high current intensities.	The application of micro-current is able to modulate the synthesis of collagen. In particular, in dependency of the current magnitude collagen type I can be up- or down-regulated. Collagen type I is responsible for the stiffness and the degree of dilatation of the heart. Therefore it can be envisaged that this method - if applied clinically - may help to improve cardiac function	Customized	Current of different magnitudes (0, 20, 40, 60, 80, 100 µA)
107	Johannes Mueller,1 Barbara Kapeller,2 Udo Losert,2 Karin Macfelda.	2006	Austria/Germany	doi:10.1016/j.clim.2006.04.299	Mueller, Johannes & Kapeller, Barbara & Losert, Udo & Macfelda, Karin. (2006). Sa.67. Electrical Microcurrent Application Modifies the Inflammatory Response in the Failing Myocardium. Clinical Immunology - CLIN IMMUNOL. 119. 10.1016/j.clim.2006.04.299.	In vitro study	Cardiomyocytes of adult rats	To investigate the effect of electrical microcurrent on cardiomyocytes and the inflammatory status under culture conditions.	Changes in the cell proliferation; collagen I, III; MMP 2,3,8,9,13,14,16; TIMP 1,2; IL-1h,6; TNF-a; TGF-h; GM-CSF; connexin 40,43,45 were measured.	1.After 90 h of cultivation, compared to the control cultured cardiomyocytes proliferation increased by 70% under low microcurrent application. 2. Collagen I synthesis decreased by 35% (low microcurrent) and collagen III increased by 100% (high microcurrent). 3.The MMPs (3,8,9,16) expression showed a significant decrease at high microcurrent application. The TIMPs remained unaffected. 4. IL-6 was suppressed by high microcurrent. 5. TNF-a, IL- 1h and GM-CSF were not detected at all.	Microcurrent application on cardiomyocytes can influence the inflammatory status, other components and dependent cellular processes of the extracellular matrix significantly by its amplitude. So it may be conceivable to influence the affected extracellular matrix by electrical microcurrent to normalize their func- tion in patients with heart failure which remains to be verified in pre-clinical and clinical trials	Customized	Low (50 µA) and high (100 µA) MC
108	Johannes H Mueller,1 Barbara Kapeller,2 Udo M Losert,2 Karin Macfelda.2	2006	Germany/Austria	doi:10.1016/j.healun.2005.11.140	Mueller, J., Kapeller, B., & Macfelda, K. (2006). 134. The Journal of Heart and Lung Transplantation, 25(2), S90. doi:10.1016/j.healun.2005.11.140	In vitro study	Cardiomyocytes of adult rats	Mueller et al. (127) looked at the effects of microcurrent on healthy and heart failure cardiac tissue (cardiomyocytes from SHR were compared to tissue samples from Wistar Kyoto rats (WT)) before and after microcurrent application. SHR tissue was not treated with microcurrent and acted as a control group, while cardiomyocytes from wild-type rats were not treated with microcurrent.	Cardiomyocytes from spontaneously hypertensive rats (SHR) were compared to tissue samples from Wistar Kyoto rats (WT) before and after application of low and high (50µA vs 100 µA) MC. Tissue from SHR rats without any MC application served as reference. Tissue samples from WT rats were not exposed to MC. Changes in collagen I, III; in MMP 2, 3, 8, 9, 13, 14, 16; TIMP 1–4 and connexin 40, 43, 45 were qualified and quantified	1. PCR-Analysis showed characteristic expression patterns for healthy and diseased cardiac tissue. 2. Most differences were shown in collagen I synthesis (plus 45% in diseased tissue) as well as in MMP 9, 13, 14 (plus 87% in diseased tissue) and TIMP 2 (minus 54% in diseased tissue).	MC influences the composition of cardiac ECM by normalizing collagen and degradation processes. This method built a basis for clin- ical application and can be envisioned to provide assistance in improve- ment of cardiac function in humans.	Customized	Low (50 µA) and high (100 µA) MC
112	Barbara Kapeller1*, Johannes Mueller2, Udo Losert1, Bruno K. Podesser1 and Karin Macfelda1	2016	Austria/Germany	doi: 10.1002/ehf2.12080	Kapeller B, Mueller J, Losert U, Podesser BK, Macfelda K. Microcurrent stimulation promotes reverse remodelling in cardiomyocytes. ESC Hear Fail. 2016;3(2):122–30.	In vitro study	Cardiomyocytes	If the application of microcurrent (MC) can modulate the expression of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) in cardiomyocytes in vitro and in vivo to reverse remodelling in the heart in spontaneous hypertensive rats (SHR).	Cardiomyocytes from young SHR (7 months) and old SHR (14 months) were stimulated in vitro and in vivo with MC. MMP and TIMP expression were analysed by qPCR and immunofluorescence to evaluate the modulation of MC treatment	1. Modulation of cardiomyocytes with MC enhances proliferation with no morphological changes in vitro. 2. Microcurrent has showed multiple effects, an increase and a decrease, on MMP-2, MMP-9, TIMP-3, and TIMP-4 mRNA and protein expression, depending on the cardiomyocytes' age. 3. Young SHR had higher levels of MMP-2, MMP-9, and TIMP-4, as well as higher levels of TIMP-3. MMP-2, MMP-9, and TIMP-4 were up-regulated in the elderly SHR, while TIMP-3 was unaffected	The data indicates that treatment of MC can modulate the expression of MMPs and TIMPs in vitro and in vivo in SHR. Based on these results new treatments for heart failure could be developed	Customized	Direct MC (~1 µA)
102	Aida Llucià-Valldeperas, Benjamin Sanchez, Carolina Soler-Botija, Carolina Gálvez-Montón, Santiago Roura, Cristina Prat-Vidal, Isaac Perea-Gil, Javier Rosell-Ferrer, Ramon Bragos and Antoni Bayes-Genis	2014	Spain	doi:10.1186/scrt482	Llucià-Valldeperas A, Sanchez B, Soler-Botija C, Gálvez-Montón C, Roura S, Prat-Vidal C, et al. Physiological conditioning by electric field stimulation promotes cardiomyogenic gene expression in human cardiomyocyte progenitor cells. Stem Cell Res Ther. 2014;5(4):1–5.	In vitro study	Cardiomyocyte progenitor cells (CMPCs) isolated from human adult atrial appendages	Electrical conditioning of cardiomyocyte progenitor cells (CMPCs) might enrich their cardiovascular potential.	Llucià-Valldeperas et al. used a custom-designed stimulation unit for the electrical conditioning of cardiomyocyte progenitor cells (CMPCs). It comprised a monophasic programmable electrical device, together with an electrical isolation stage and two printed circuit boards, which allowed the fast and robust connection of the electrodes. A biocompatible polydimethylsiloxane silicone-patterned system was designed to provide structural support to cells and electrodes. Their protocol has been comprised of 30,000 seeded cells to 2-ms monophasic square-wave pulses of 25 mV/cm at 1 Hz (alternating current) for 7 and 14 days	1. GATA-binding protein 4 (GATA4) early transcription factor was significantly overexpressed (P =0.008), 2. Coactivator myocyte enhancer factor 2A (MEF2A) was upregulated (P = 0.073) under electrical stimulation. 3. Important structural proteins and calcium handling-related genes were enhanced.	Electrostimulated CMPCs may be best-equipped for myocardial integration after transplantation.	Customized	30,000 seeded cells to 2ms monophasic square-wave pulses of 25 mV/cm at 1 Hz (alternating current) for 7 and 14 days

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
9	104	L. Wen C. Zhang Y. Nong Q. Yao (&) Z. Song	2013	China	doi:10.1007/s12013-012-9402-x	Wen L, Zhang C, Nong Y, Yao Q, Song Z. Mild Electrical Pulse Current Stimulation Upregulates S100A4 and Promotes Cardiogenesis in MSC and Cardiac Myocytes Coculture Monolayer. Cell Biochem Biophys. 2013;65(1):43–55.	In vitro study	Canine mesenchymal stem cells	Based on the injury current theory, Wen et al. used electrical microcurrent cardiomyocyte stimulation of MSCs and newborn rat cardiomyocytes to investigate cardiogenesis and angiogenesis	Wen et. al. used a program-controlled machine (not further specified) to stimulate canine mesenchymal stem cells (CMSCs) and cardiac myocytes with an electrical impulse which is rectangular 2 ms, 2 Hz and 40 μA of strength. MCS and cardiac myocytes have been prepared in uniform suspension. A 100 kΩ was added to eliminate the waveform change caused by the electric capacity of the culture system. A platinum wire was used as an electrode placed on culture dish cover (corning). Electric signal treatment was given 1, 3, and 6 h/day for 4 days s	1. CD44 was reduced in the MSCs monolayer treated with EPCS, compared with non-stimulated MSCs; and 2. EPCS MSCs (3 h/day, 6 h/day, 5 days) showed an 14.04 ± 3.44 and 14.55 ± 3.97 % reduction in CD44, compared with the cotemporary MSCs; these reveal that CD44 reduction amplitude is not correlated with time for EPCS disposure and CD29 (integrin b1) expression is not affected by EPCS exposure. 3. EPCSwas given to the MSCs and cardiacmyocytes coculture monolayer (ratio 3:1) for different time (1, 3, and 6 h/day) for 4 days to see the biological effects. Gap junction protein and troponin T show an increase after EPCS. We found that the gap junction protein Cx43 increased with treating time—in the EPCSgroup, it exhibited 1.5 and 1.7 fold in the 3 h/day group and 6 h/day group (P\0.01), and troponin T exhibited to about 3.6 and 4.4 fold in the 3 h/day group (P\0.01) and 6 h/day group (P\0.05). Since coculture was used as stimuli, immunofluorescence was used to visualize the changes during EPCS for the purpose of elu- cidating the impact of EPCS on cardiac myocytes and MSCs 4. After 5 days exposure, EPCS can enhance the expression of S100A4, which is 2.33 fold in cardiac myocytes (P\0.01) and 1.99 fold inMSCs (P\0.01) in gray value. A significant increasing expression of the myocyte enhancer factor (MEF) and GATA4 is detected in neonatal rat cardiac myocytes (P\0.01) compared with cotemporary coculture monolayer in the control group. 5. Also, EPCS can trigger the assembly of MEF2c in the nuclei. In addition, more cardiac myocytes were found to have two nuclei. 6. But MSCs fail to active MEF2C transcriptional factor like that in cardiac myocytes after EPCS exposure. The elevation of MEF2 in both cytoplasm and nuclei of cardiac myocytes can always make a clear distinction of the cardiac myocytes and MSCs in coculture.	Pulsed microcurrent stimulates cardiogenesis and angiogenesis by increasing S100A4 in MSCs and cardiac myocytes as well as supports cardiac myocyte development (upregulation of MEF2C and GATA4, Cx43 and troponin T).	Customized	Pulse polarity altered one after another, rectangular 2 ms, 2Hz, 40 μA A 100 kX electric resistance was added to the circuit in series connection to eliminate the waveform change caused by the electric capacity ofculture system
10	110	Karin Macfelda1, Alexander Holly1, Johannes Mueller2;	2015	Germany/ Austria	doi:10.1016/j.cardfail.2015.06.091	Macfelda K, Holly A, Mueller J. Significant Enhancement of ATP-Synthesis in Cardiomyocytes By Electric Microcurrent. J Card Fail [Internet]. 2015;21(8):S19. Available from: http://dx.doi.org/10.1016/j.cardfail.2015.06.091	In vitro study	Cardiomyocytes of the myocardium of spontaneous hypertensive rats (SHR)	Macfelda et al. (129) used microcurrent to stimulate SHR cardiomyocytes in culture, with the goal of determining the mitochondrial respiration rate and ATP generation after electrical stimulation	Cardiomyocytes of the myocardium of spontaneous hypertensive rats (N55; 11 weeks old) were stimulated electrically by use of a direct current (dc) power generator via two electrodes under cultured conditions. Of each SHR myocardium, five times three specimens of myocytes were taken, cultured and exposed to the dc with the intensity zero (control), 10 (low) or 100 (high) mA over a period of 72h. Mitochondrial respiration of dc treated cells was measured via the Oxygraph 2K (Oroboros, Innsbruck, Austria). This instrument allows the continuous measurement of oxygen consumption of intact cells. A sequence of the inhibitors oligomycin, carbonyl cyanide-4-(trifluor-omethoxy)phenylhydrazone and rotenone was added to analyze the impact of different mitochondrial complexes on respiration. ATP of microcurrent treated SHR cardiomyocytes was isolated by an ATP-assay (Abcam, ab83355) and quantified fluorometricall	1. In comparison to the control group, she detected a small upregulation of mitochondrial respiration with 10 μA (+ 28.6%) and a considerable upregulation with 100 μA (+ 45.4%) microcurrent treated cells. 2. With 10 μA (+ 8.5%) and 100 μA (+ 16.7%) microcurrent stimulation, there was a slight increase in ATPase efficiency. 3. When compared to the control group, the results show a considerable increase in ATP of 98.4% (+/- 26.7 percent) with 10 μA and 172.3 % (+/-41.7 percent) with 100 μA treated cells.	The data obtained in this experiment suggests that microcurrent application increases cell respiration and ATP-synthesis. This may be potentially relevant to the treatment of heart failure. 022	Customized	Direct current with the intensity zero (control), 10 (low) or 100 (high) μA over a period of 72h
11	111	Karin Macfelda1*, Barbara Kapeller1, Alexander Holly1, Bruno K. Podesser1, Udo Losert1, Kersten Brandes2, Peter Goettel2 and Johannes Mueller2*	2017	Germany/ Austria	doi: 10.1002/ehf2.12169	Macfelda K, Kapeller B, Holly A, Podesser BK, Losert U, Brandes K, et al. Bioelectrical signals improve cardiac function and modify gene expression of extracellular matrix components. ESC Hear Fail. 2017;4(3):291–300.	In vivo study	16 hearts of male spontaneously hypertensive rats (SHRs)	Macfelda (130) applied microcurrent to heart failure SHR and investigated whether applying electrical microcurrent directly to failing hearts modifies gene expression of extracellular matrix components and improves cardiac functionality	Sixteen male spontaneously hypertensive rats (SHRs) with heart failure underwent application of a patch electrode to the left ventricular epicardium and placement of a subcutaneous counter electrode. The electrode delivered a 0.35 μA microcurrent to nine of the SHRs for 45 ± 3 days; the other seven SHRs were used as controls. At baseline and before the SHRs were humanely put to death, we measured the left ventricular ejection fraction (LVEF) and the thickness of the LV posterior wall during systole and diastole (LVPWs/d). We used quantitative PCR to determine extracellular matrix parameters [collagen I–III, matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinases 3 (TIMP3), TIMP4, connexins (Cxs) 40/43/45, transforming growth factor (TGF)-β, and interleukin (IL)-6].	1. Echocardiographically, microcurrent application exhibited a statistically significant normalization of the LVEF and a significant decrease in the thickness of the LV posterior wall during systole and diastole (LVPWs/d) – (LVPWs (mean decrease, 35.3%, LVPWd mean decrease, 20%). The control group showed no changes. 2. Statistically significant measurements of stimulated SHR hearts reveled a down-regulation in the expression of collagen type I (10.6%), TIMP3 (18.4%), Cx43 (14.3%), Cx45 (12.7%), TGF-β (13.0%), and IL-6 (53.7%). 3. No statistically significant changes after microcurrent application could be seen in the downregulation of collagen type III (1.4%), MMP-2 (1.7%), MMP-9 (2.8%), TIMP4 (5.2%), or Cx40 (4.7%).	Long-time and direct in vivo application of microcurrent to the epicardium of beating hearts of SHRs with heart failure leads to statistically significant alterations in the gene expression of ECM components, including collagen type I, TIMP3, Cx43, and Cx45. Additionally, the use of current reduces the levels of the cytokines IL-6 and TGF-ß.	Customized	Direct microcurrent of 0.35 μA for 8.5 h daily over a mean period of 45 ± 3 days.
12	114	Mueller1, B. Kapeller2, H. Heinze1, M. Hofmann2, J. Holfeld2, U.M. Losert2, K. Macfelda21	2009	Germany/ Austria	doi:10.1016/j.healun.2008.11.812	Mueller J, Kapeller B, Heinze H, Hofmann M, Holfeld J, Losert UM, et al. 134: Does the Direct Myocardial Application of Electrical Microcurrent Heal Heart Failure by Downregulation of the Pro-Inflammatory Cytokines? J Hear Lung Transplant [Internet]. 2009;28(2):S112.	In vivo study	5 hearts of male spontaneously hypertensive rats (SHRs)	Mueller et al. (133) investigated the effect of electrical microcurrent on the ECM of the SHR heart compared to wild-type rats (WKY)	MC was applied over a period of up to 35 days via two epicardial patch electrodes to the myocardium of 5 SHR. Mean values of the measured parameters of the myocardial ECM were analysed and compared to those from 5 wild type rats (WKY) and 5 SHR without previous MC application. Gene expression (quantitative PCR) was measured for MMP-2,9; TIMP-3,4; connexin 40,43,45; collagen I,III and IL-6 and TGF-ϒ	1. Compared to the control group (5 WKY and 5 SHR), microcurrent application to the myocardium of the 5 SHR up-regulated the level of MMP-2,9 and downregulated the level of TIMP-3 significantly. 2. TIMP-4 remained unchanged. 3. Expression for collagen I did not change, but collagen III was significantly reduced (> 40%). 4. Parameters for myocyte vitality, connexin 40, were up-regulated by > 30%. 5. Microcurrent reduced the expression of IL-6 as well as TGF-ß as compared to the native myocardium of SHR and WKY rats	MC regulates MMPs and collagen III on the gene level and normalizes the ECM. Interestingly, TGF-ß and IL-6 were significantly downregulated. Connexin 40 as parameter of cell vitality showed an increase in the gene expression pattern. Thus, MC application initializes a process towards healing of the diseased myocardium	Customized	MC - not further specified

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
13	115	J.H. Mueller,1 B. Kappeler,2 M. Hofmann,2 U.M. Losert,2 K. Macfelda,21	2008	Germany/ Austria	doi:10.1016/j.healun.2007.11.443	Mueller JH, Kappeler B, Hofmann M, Losert UM, Macfelda K. 431: Does the Application of Electrical Microcurrent Heal Heart Failure? First Pre-Clinical In Vivo Results. J Hear Lung Transplant. 2008;27(2):S216.	In vivo study	Hearts of male spontaneously hypertensive rats (SHRs)	The goal of these in-vivo experiments is to investigate the effect of electrical microcurrent on the ECM and as a consequence on healing of heart failure. As previously published, MC is able to modify the myocardium (collagen, MMPs, TIMPs) of spontaneously hypertensive rats (SHR) under in-vitro conditions	Two plane electrodes were surgically placed around the heart of SHR covering the right and the left heart's epicardium. MC was applied over a period of 5 days. Thereafter, parameter of the ECM of the myocardium was analysed and compared to the myocardium from wild type rats (WKY) and SHR without previous MC application. Gene expression (quantitative PCR) was measured for MMP 2, 3, 8, 9, 13, 14, 16; TIMP 1, 2, 3, 4; connexin 40, 43, 45 and collagen I and III and eventually the level of IL-6	1. Compared to the myocardium of WKY, the myocardium of SHR without MC application showed a significantly higher level of MMP 3, significant lower level of MMP 8, 14, 16 and an unchanged level of MMP 2 and 13. 2. The TIMPs and connexins were only marginally altered. Collagen I showed an upregulation of 40 %. After MC application, MMP 2, 3, 9, 13, 14 and 16 were significantly up-regulated, MMP 8 remained unchanged, and most importantly, collagen I up-regulated by a factor of 2.5. 3. The gene expression level of IL-6 was significantly downregulated	MC application regulates MMPs as well as collagen I on the gene level and normalizes the ECM of hearts in a progressed state of failure. Interestingly, the proinflammatory IL-6 was significantly downregulated. MC application initializes a process towards healing of the diseased myocardium. 432	Customized	MC - not further specified
14	116	s H. Mueller1, Barbara Kapeller2, Michael Hofmann2, Udo M. Losert2, Karin Macfelda2;	2007	Germany/ Austria	doi:10.1016/j.cardfail.2007.06.465	Mueller JH, Kapeller B, Hofmann M, Losert UM, Macfelda K. Myocardial Extracellular Matrix Normalization by Electrical Microcurrent Application In Vivo. Basics for a Novel Heart Failure Treatment. J Card Fail. 2007;13(6):S119.	In vivo study	Hearts of male spontaneously hypertensive rats (SHRs)	The goal of these in-vivo experiments is to investigate the effect of electrical microcurrent on the improvement of heart failure with the primary focus on the myocardial extracellular matrix.	MC application consisted of implanting SHR with two electrodes into the myocardium (lateral wall of the left ventricle). MC was applied over a period of 5 days. The animals were then killed, and the myocardium analysed and compared to the myocardium from wild type rats (WKY) and SHR without previous MC appli- cation. Additionally, for direct reading the MC effect, the areas of myocardium which were not exposed to MC (outside of the current flow) was compared to the MC-exposed myocardium of the same heart. Gene expression (PCR) was measured for MMP 2, 3, 8, 9, 13, 14, 16; TIMP 1, 2, 3, 4; connexin 40, 43, 45 and collagen I and III	1. After microcurrent application of SHR, MMP 2, 3, 8, 9, 13, 14 and 16 were significantly upregulated, as well as collagen I, which was upregulated towards the level of collagen I of the control WKY group. 2. Comparing the effects inside and outside the current flow to the myocardium, confirmed the results of microcurrent use in comparison with the WKY control group	MC application up-regulates MMPs as well as collagen I on the gene expression level and normalizes the extracellular matrix of hearts in a progressed state of failure	Customized	MC - not further specified
15	109	J. Mueller,1 T. Giesel,1 T. Toellner,1 K. Brandes,1 B. Kapeller,2 A. Strautmann,1 A. Prehn,1 R. Alnajjar,1 B. K. Podesser,2 A. Kramer,2 P. Goettel,1 K. Macfelda	2017	Germany/ Austria	not found	Mueller JH. ABSTRACTS ASAIO 63 rd ANNUAL CONFERENCE Chronic Administration of Electrical Microcurrent to the Heart is Safe and Does Not Impair Cardiac Function ABSTRACTS ASAIO 63 rd ANNUAL CONFERENCE CHICAGO , IL. (June 2017)	In vivo study	5 hearts of healthy sheeps	To assess safety margins for further clinical studies of microcurrent application on healthy sheeps	Blood chemistry and blood cell count parameters, LVEF and fractional shortening (FS) were reported 12 times, performed by echocardiography, and were assessed statistically by non-inferiority testing	1. The mean LVEF increased by 1.4 % from 71.4 to 72.8 % which was not significant. 2. The probability to impair the LVEF through EMC by more than 3.9% within 6 months is less than 5 %. 3. The mean FS rose by 4.6 % from 44.8 to 49.4% which just missed statistical significance (P=0.068). 4. Alterations of the ECG not reported, all blood chemistry and cell count parameters stayed in the normal ranges	The chronic administration of EMC of 5µA per cm2 to hearts of healthy sheep is safe and does not compromise heart function	Customized	MC of 5µA per cm2, 180 µA over a period of 195 days
16	117	L. Christian Napp,1 Guenes Dogan,1 Jasmin Hanke,1 Silvia Mariani,1 Peter Göttel,2 Kersten Brandes,2 Samir Sarikouch,1 Christoph Bara,1 Johannes Müller,2 Jan Schmitto	2019	Germany/ Switzerland	doi:10.1016/j.jacc.2019.08.419	Napp LC, Dogan G, Hanke J, Mariani S, Göttel P, Brandes K, et al. TCT-338 Electrical Microcurrent Therapy for Treatment of Chronic Systolic Heart Failure: Animal and First-in-Man Data. J Am Coll Cardiol [Internet]. 2019;74(13):B335.	In vivo study	12 hearts of male sheeps	Napp et. al. (136) investigated the chronic application of a microcurrent by using the C-MIC device from Berlin Heals in a sheep model of chronic heart failure inducing reverse remodeling and modifying myocardial inflammation	12 sheep were subjected to implantation of a novel medical device (C-MIC), which consists of an extremely thin patch electrode mounted to the epicardium of the left ventricle (LV), a transvenous counter lead electrode in the right ventricle, and a subcu- taneous microcurrent generator connected to both leads. First, the surgical procedureand the electrode design were optimized in 5 sheep, and thereafter HF was induced by in- jection of 90-mm microspheres directly into the left coronary artery in the remaining 7 sheep. Coronary microembolization was repeated until a sustained reduction of LV ejection fraction (EF) was achieved, defined as a stable mean reduction by 25% on echocardiography. Thereafter, the device was activated to continuously apply a transmyocardial microcurrent to the failing heart. Echocardiography was repeatedly performed to measure LVEF during microcurrent treatment until 12 weeks after activation of the device.	1. After coronary embolization in sheep, LVEF strongly decreased (baseline: 70.9 ± 5.0% after embolization: 44.2 ± 13.4%; p < 0.001) 2. Within 12 weeks after activation of the microcurrent device, therapy was associated with improvement of the LVEF back to 62.4 ± 7.3% (p ¼ 0.206 as compared to baseline), consistent with a mean relative increase of 68% 3. Adverse events like intraoperative mortality, bleeding or re-thoracotomy did not occur throughout the whole study period.	Continuous microcurrent application seems safe and improves LVEF in sheep chronic systolic HF models.	C-MIC	MC not further specified
17	103	Ping Zhang Zhi-Tao Liu Guo-Xiang He Jian-Ping Liu Jian Feng	2011	China	doi:10.1007/s12013-010-9107-y	Zhang P, Liu ZT, He GX, Liu JP, Feng J. Low-Voltage Direct-Current Stimulation is Safe and Promotes Angiogenesis in Rabbits with Myocardial Infarction. Cell Biochem Biophys. 2011;59(1):19–27.	In vivo study	30 Japanese white rabbits hearts	To evaluate the safety and efficacy of low-voltage direct-current (DC) electrical stimulation of angiogenesis in rabbits with myocardial infarction (MI).	Zhang et. al. used a direct current power supply (Shan-Jie Technological Limited Company, Shanghai, China) for the electrical stimulation of rabbit hearts after ligation of the LCX (left circumflex artery). Electrodes have been sutured to the epicardium intraoperatively and where then running through a subcutaneous tunnel on the rabbits neck. Low-voltage direct current microcurrent stimulation (EF of 4.0 V/cm for 30 min) was applied right after surgery and continued until the fourth post-operative week	1. Immunohistochemical study using anti-factor VIII antibody to stain endothelial cells showed that capillary density in the treatment group (63.1 ± 2.2) was significantly higher (P\0.01) than that of control group (45.4 ± 3.9). 2. An inflammatory response to the increase was excluded (no significant changes in monocyte chemotactic protein-1 (MCP-1) expression between treatment and control groups). 3. Regarding electrical stimulation, no adverse reactions except a minor infiltration of inflammatory cells and mild degeneration were observed in the myocardium.	Electrical stimulation directly applied to the heart effectively induces and/or promotes angiogenesis in MI-induced rabbit hearts.	Customized	Low-voltage DC stimulation (electric field of 4.0 V/cm for 30 min/day)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
18	105	Rupak Mukherjee, William T. Rivers, Jean Marie Ruddy, Robert G. Matthews, Christine N. Koval, Rebecca A. Plyler, Eileen I. Chang, Risha K. Patel, Christine B. Kern, Robert E. Stroud, and Francis G. Spinale	2010	USA	doi: 10.1161/CIRCULATIONAHA.110.936872.Chronic	Mukherjee R, Rivers WT, Ruddy JM, et al. Long-term localized high-frequency electric stimulation within the myocardial infarct: effects on matrix metalloproteinases and regional remodeling. Circulation. 2010;122(1):20-32	In vivo study with in vitro fibroblast stimulation	17 hearts of mature pigs	R.Mukherjee et al. (124) tried to use a targeted approach through localized high frequency stimulation (LHFS) of pig hearts in vivo, using low amplitude electrical pulses inserted within an idiopathically formed MI scar to alter MMP/TIMP levels and to prevent MI thinning	R.Mukherjee et al. used a more complex approach by first inducing an MI in mature pigs by ligation of the LCX, and then using a pacemaker to confine electrical activation to within the MI region and additionally analyze electrically stimulated fibroblasts. The parameters used for chronic localized high frequency stimulation have been chosen to be activated at 240 bpm using low amplitude and short duration pulses (VOO mode, 0.8V, 0.05 ms). After 28 days post-MI, the pacemaker was deactivated and regional LV dimensions and wall thickness were measured, as well as systemic and pulmonary artery pressures, cardiac output, and LV pressures. Furthermore, fibroblasts have been isolated from the LV free wall of non-MI pigs. They were electrically stimulated using microcurrent of 5 ms, 2 A, and 4 V/cm pulses of alternating polarity for 24 hours. The cells were stimulated at either 4 Hz or left unstimulated (0 Hz)	1. All 17 pigs entered into the study survived the initial instrumentation and MI induction. 2. LV echocardiographic measurements recorded for the control pigs and at post-MI days 21 and 28 for the two MI groups are presented in Table 1. 3. LV posterior wall thickness was reduced and septal wall thickness was increased at 21 days post-MI for the MI group that was left unstimulated (UNSTIM) or for the MI group with low amplitude, high frequency stimulation (LHFS), with no difference between groups. 4. At 28 days post-MI, LV posterior wall thickness was reduced further in the UNSTIM group, but remained similar to 21 day values in the LHFS group. 5. The change in LV end-diastolic volume from 21 days post-MI to 28 day post-MI was lower with LHFS compared to the UNSTIM group (3.2±2.6% vs. 12.9±5.3%, respectively, p=0.03). 6. LV ejection fraction in the post-MI period was similar in both groups	The main result was that LHFS attenuated MI thinning and increased regional stiffness of the MI region. Also, LHFS downscaled the levels of certain MMP subtypes and raised TIMP-1 levels, which were joined by enhanced collagen content in the MI region. Also noteworthy is that electrical stimulation of myocardial fibroblasts, extracted from the stimulated pig hearts, revealed a reduction of specific MMP types and an increase in TIMP-1 levels. These findings provide evidence that subthreshold electrical stimulation of the MI region may represent a novel means to inhibit and/or even prevent adverse LV remodeling post-MI.	Customized	5 ms, 2 mA, 4 V/cm pulses of alternating polarity in each chamber. The cells were stimulated at either 4 Hz or left unstimulated (0 Hz) for 24 hours
19	118	S. Mariani, C. Napp, T. Meyer, K. Brandes, C. Baum, P. Göttel, J.S. Hanke, A. Merzah, M. Al Masarani, L. Tong, A. Haverich, G. Dogan, J. Müller, and J.D. Schmitto	2020	Germany	doi: 10.1016/j.healun.2020.01.436	1. Mariani S, Napp C, Meyer T, Brandes K, Baum C, Göttel P, et al. Animal Model of Cardiac Reperfusion Injury to Evaluate the Effects of Electrical Microcurrent Application: Preliminary Results. J Hear Lung Transplant. 2020;39(4):S361.	In vivo study	7 hearts of mature pigs	Mariani et al. (137) tried to investigate whether microcurrent application using the C-MIC device can modulate cardiac reperfusion injury (RI) after cardiopulmonary bypass (CPB)	Seven swines underwent CPB with 120 mins crossclamp time, and were assigned to a control (n=3) or treatment group (n=4). The treat- ment group received a C-MIC device consisting of an epicardial patch electrode placed on the left ventricular lateral free wall, a transvenous right ventricular counter electrode, and a current generator. C-MIC treatment was continued until CPB weaning. Reperfusion on full CPB flow lasted 20 mins, followed by a stepwise weaning process. Inotropes or pacing were not allowed. Noradrenaline was used to maintain arterial pressure and xylocaine for arrhythmia treatment. Defibrillation was performed as needed. Primary end-point was survival at 20 mins after CPB weaning.	1. Reperfusion on full CPB flow lasted 20 mins. This process was followed by a stepwise weaning procedure. 2. The primary endpoint was survival at 20 mins after CPB weaning. Results in the C-MIC group are successful CBP weaning in all animals and survival with stable hemodynamics in 50% of cases. 3. Weaning and survival rates were lower in the control group. The data, shown in figure 16, showed the potential benefit of C-MIC treatment for RI. **Table 1** Operative data | Variable | C-MIC (n=4) | Control (n=3) | | --- | --- | --- | | Weight (Kg) | 86.5 (84-87) | 84 (84-90) | | Operation Time(min) | 318 (302.5-342.5) | 268 (261-313.5) | | Total CPB time (min) | 191.5 (162-220) | 146 (145.5-160.5) | | DC shock (n) | 7 (4-13.5) | 9 (6.5-10.5) | | MAP end-CPB (mmHg) | 34.5 (30-41.5) | 28.5 (20-37) | | Survival (n,%) | 2, 50% | 1, 33% | | CPB weaning (n%) | 4, 100%) | 2, 67%) |	The study presents an animal model which allows evaluation of RI treatment strategies. In this pilot trial, the C-MIC device was safely and successfully implanted. Our data suggest a potential benefit of C-MIC treatment for RI and further studies are needed to test this promising therapy	C-MIC	Not mentioned
20	113	Jesus Eduardo Rame, MD, MPhil,1 and Johannes Mu¨ ller, MD, Meng	2021	USA/Germany	doi: 10.1089/bioe.2021.0021	Rame JE, Müller J. Myocardial edema revisited in a new paradigm of cardiac electrical microcurrent application in heart failure. Bioelectricity. 2021;3(3):171–5.	Translational study	Hearts of male sheeps	To discuss whether the direct application of an external electric current (in the physiological IA range) together with an electrical field to hearts with impaired pump function can explain the functional improvement of the hearts by edema reduction triggered by electro-osmosis	Translational work: Proportionality of electrical current application and cardiac output. In a sheep model with surgically implanted electrodes and measuring cardiac output through a CCO monitoring system (Vigilance; Baxter Healthcare Corporation's Edwards Critical-Care Division), we demonstrate the time correlation of microcurrent application and cardiac output. In this model, an increase in microcurrent is paralleled by an increase in cardiac output. Given the immediate stroke volume response, a mechanism that could explain the impact of microcurrent on the myocardium is a reduction in myocardial fluid content. Consistent with this observation is that a reduction in microcurrent does not lead to an immediate drop in CCO because the process of fluid retention is subject to a physiological delay of reaccumulation of fluid into the myocardium	1. An externally applied weak electric current together with an electrical field can interrupt the vicious circle of edema formation and impaired cardiac performance. It normalizes the electrical environment with the consequence of increasing lymphatic flow by supporting the electrokinetically induced transport of electro-osmosis and electrophoresis and may also influence the charge of the glycocalyx. 2. Without prejudging clinical confirmation, one could hypothesize that the spectrum of patients with heart failure who benefit from the application of microcurrent directly to the heart may be largely independent of the cause of the disease, depending only on the extent of myocardial edema.	The early improvement in cardiac function after the application of a microcurrent directly to and around the heart could implicate favorable changes in myocardial edema, which heretofore has not been a clinical target in human heart failure	C-MIC likely	MC - not further specified
21	94	Jan D. Schmitto,* L. chriStian napp ,† SiLvia mariani ,* JaSmin S. hanke,* tong Li,* JenS vogeL-cLauSSen,‡ kerSten BranDeS,§ peter göttel,§ JohanneS mülLer,§ gueneS Dogan,* Johann BauerSachS ,† anD axeL haverich*	2021	Germany	doi:10.1097/mat.0000000000001537	Schmitto JD, Napp LC, Mariani S, Hanke JS, Li T, Vogel-Claussen J, et al. First-in-man Implantation of a Cardiac Microcurrent Device for Chronic Systolic Heart Failure. ASAIO J. 2021;Publish Ah:1–3.	Case report	Human heart	First-in-man experience with the implantable device for cardiac electrical microcurrent (C-MIC) application	Implantation was conducted under general anesthesia with a minimally invasive surgical approach. A 5 cm incision was performed at the level of the left infraclavicular fossa, and the LV lead was tunneled subcu- taneously from the left pleural cavity to the new prepectoral pocket. The left subclavian vein was punctured, and the RV lead implanted in the RV cavity under fluoroscopic guidance. Both LV and RV leads were connected to the C-MIC generator, which was finally implanted in the prepectoral pocket. Wounds were closed, and correct position of the device and leads was confirmed by fluoroscopy	1. The C-MIC device continuously operated without malfunction, and no electrical interference between devices was observed during hospitalization and at 30-day follow-up. | Variables | Preimplant | Discharge | 30 days | | --- | --- | --- | --- | | Vital signs | | | | | Heart rate, beats/min | 57 | 80 | 55 | | Systolic blood pressure, mm Hg | 141 | 140 | 113 | | Diastolic blood pressure, mm Hg | 53 | 83 | 53 | | Echocardiogram | | | | | LVEF, % | 30 | 35 | 40 | | LVEDD, mm | 6.63 | 6.45 | 6.6 | | LVESD, mm | 5.37 | 5.56 | 5.34 | | PAPs, mm Hg | 32 | | 28 | | Laboratory data | | | | | White blood cells, x1,000/mL | 13.5 | 10.6 | 9.0 | | Hemoglobin, g/dL | 12.6 | 11.3 | 9.9 | | Platelets, x1,000/mL | 176 | 179 | 230 | | AST, U/L | 29 | n.a. | 21 | | ALT, U/L | 31 | n.a. | 12 | | Creatinine, µmol/L | 134 | 175 | 142 | | CK, U/L | 24 | 37 | 31 | | NT-proBNP, pg/mL | 4,805 | n.a. | 4,529 |	Although this first-in-man use and preclinical data indicate possible improvements of systolic cardiac function, a prospective study is required to assess C-MIC efficacy in HFrEF patiens with persisting symptoms with optimal therapy	C-MIC	Continuous direct transmyocardia microcurrent (DC current) in the µ-ampere range, which is applied independently from electrical or mechanical cardiac activity

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
22	92	Dragana Kosevic1, Dominik Wiedemann2 , Petar Vukovic1 , Velibor Ristic1, Julia Riebandt2 , Una Radak1, Kersten Brandes3, Peter Goettel3 , Hans-Dirk Duengen4, Elvis Tahirovic5, Tatjana Kottmann6, Hans Werner Voss6, Marija Zdravkovic7 , Svetozar Putnik8, Jan D. Schmitto9, Johannes Mueller3*	2021	Austria/Germany/Serbia/USA	doi: 10.1002/ehf2.13242	Kosevic D, Wiedemann D, Vukovic P, Ristic V, Riebandt J, Radak U, et al. Cardio-microcurrent device for chronic heart failure: first-in-human clinical study. ESC Hear Fail. 2021;8(2):962–70.	Clinical trial - phase I	Humans (first non-randomized pilot study)	To investigate whether chronic application of electrical microcurrent to the heart is feasible and safe and improves cardiac performance	This single-arm, non-randomized pilot study involved 10 patients (9 men; mean age, 62 ± 12 years) at two sites with 6 month follow-up. All patients had New York Heart Association (NYHA) Class III heart failure and non-ischaemic dilated cardiomyopathy, with left ventricular ejection fraction (LVEF) <35%. A device was surgically placed to deliver a constant microcurrent to the heart. The following tests were performed at baseline, at hospital discharge, and at six time points during follow-up: determination of LVEF and left ventricular end-diastolic/end-systolic diameter by echocardiography; the 6 min walk test; and assessment of NYHA classification and quality of life (36-Item Short-Form Health Survey questionnaire)	1. During follow-up, rapid and significant signal of efficacy (P < 0.005) was present with improvements in LVEF, left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and distance walked. 2. For eight patients, NYHA classification improved from Class III to Class I (for seven, as early as 14 days post-operatively); for one, to Class II; and for one, to Class II/III. 3.36-Item Short-Form Health Survey questionnaire scores also improved highly significantly	Chronic application of microcurrent to the heart is feasible and safe and leads to a rapid and lasting improvement in heart function and a near normalization of heart size within days. The NYHA classification and quality of life improve just as rapidly	C-MIC	Not mentioned
23	119	Johannes Mueller, Miodrag Peric II, Dragana Bakal Kosevic, Velibor Ristic, Kersten Brandes, Peter Goettel, Petar Vukovic, Berlin	2020	Germany/Serbia	doi:10.1016/s0735-1097(20)31474-1	Mueller J, Peric M, Kosevic DB, Ristic V, Brandes K, Goettel P, et al. Electrical Microcurrent Therapy in Patients With Chronic Heart Failure: a Successful Disruptive Treatment Approach. J Am Coll Cardiol [Internet]. 2020;75(11):847. Available from: http://dx.doi.org/10.1016/S0735-1097(20)31474-1	Pilot clinical study	Humans (first non-randomized pilot study	A human pilot study, whether the application of EM directly to the left ventricular epicardium via a patch lead is safe and feasible and has a beneficial effect on cardiac function of patients (pts) with chronic heart failure (HF).	8 pts (7 male, 1 female; mean age 56.3 ± 8.5y, history of HF 2.9 ± 0.7y) were treated with optimal anti-HF medication and provided with a system (C-MIC) for the application of EM in a minimally invasive surgical procedure. A coil lead was placed transvenously in the right ventricle and a patch lead intrapericardially by lateral intercostal incision (4th intercostal space). Both leads were connected to a microcurrent generator implanted in a subcutaneous pocket at subclavian position. C-MIC was switched on at postop day 2. Pts cardiac data was monitored at postop day (d) 3 and 10 and month (m) 1 and 2. Results:	1. In all pts the components of the C-MIC system were well tolerated, no pericardial effusion was seen. 2. After EM, LVEF increased from 30.9 ± 4.2% at baseline (BL) to 38.1 ± 3.2%, 42.4 ± 5.6%, 43.3 ± 4.4% after 3d, 10d and 1m, respectively and remained on that level thereafter. 3. LVEDd dropped from 63.6 ± 3mm (BL) to 59.6 ± 3.8mm (after 3d), 58.4 ± 3.6mm (after 10d), 55 ± 2.9mm (after 2m). The mean distance patients could walk within 6 minutes increased from 186.8 ± 37.2m (BL) to 382.3 ± 42.5m (after 10d), 416.7m (after 1m) and has not changed in the further course of the study. 3. All patients were in NYHA class 3 before EM and in class 1 after 1m where they remained thereafter. 4. One patient with a left bundle branch block and a QRS complex width of 130 ms at BL showed a drop to 92 ms after 10d of EM.	This pilot study confirms for the first time the idea that patients treated with EM directly on the lateral side of LV epicardium not only reveal a pronounced improvement of cardiac function but also that the effect occurs extremely quickly after EM has been activated. EM as a method to treat HF has the potential to revolutionize HF treatment fundamentally	C-MIC	MC not further specified