On the Fabric of the Human Body

Running title: Raval et al.; On the fabric of the human heart

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With the publication of *De humani corporis fabrica* in 1543, Andreas Vesalius bestowed some of the greatest advancements of anatomic understanding since the time of Galen, correcting major misconceptions, for example the notion that the great vessels originated in the liver. It took nearly 1500 years for this evolution in anatomic thinking to transpire.

It is simultaneously humbling and invigorating to note that nearly 500 years later the study of anatomy is just as lively and full of evolutionary change. In this issue of *Circulation* Anversa and colleagues definitively dispatch the dogma of the heart as a terminally differentiated organ, a concept formulated a mere 40 years earlier, and in so doing completely recalibrate our understanding of cellular homeostasis in the healthy and diseased heart. 1

In 1973 Radovan Zak of the University of Chicago stated unequivocally that “The adult heart enlarges only by enlargement of its component muscle cells. Division of ventricular muscle cells in mammals is not activated after cardiac injury.” 2 This conclusion was largely consistent with work by Linzbach in 1960, however in this earlier comprehensive histologic analysis there was a suggestion that under certain circumstances the number of cardiac muscle fibers might increase. 3

The Anversa lab began suggesting the possibility of cardiomyocyte proliferation in 1980, 4 and by 1992 the group began to document clear evidence for replication of cardiac muscle cells in response to physiologic stress and myocardial injury in both experimental models 5-8 and humans 9, 10 These early works included such bold statements as “the recognition of factors controlling myocyte proliferation may provide a new approach for regeneration of damaged and lost myocardium, a phenomenon considered impossible for several decades.” While some recognized the power and implications of these observations 11, the Pfeffers’ call for open-mindedness often fell on deaf ears. Nevertheless, Anversa et al had opened the door to the era of
cardiovascular regenerative medicine.

Apparently unsatisfied with the disruption to the cardiovascular orthodoxy it had already caused, the Anversa lab confronted us yet again with evidence for the existence of cardiac stem cells in animals and humans. The claim now was that the mature myocyte pool was not the source of cells for senescent cell replacement, but rather that a resident bonafide stem cell population was responsible for cardiomyocyte homeostasis.

While some embraced this emerging understanding of the heart as an organ capable of repair, many remained skeptical. In 2009, however, Bergmann et al, using carbon-14 dating, documented what many felt to be unequivocal evidence of cardiomyocyte proliferation. The renewal rate of adult human cardiomyocytes was quantified using measurement of carbon-14 integration into nuclear DNA and mathematical modeling to estimate chronologic age of DNA synthesis in explanted human myocardial cells. This approach relies upon the rise and fall of radioactive atmospheric carbon-14 concentrations surrounding 1963, and the proportional incorporation of these radio-labeled molecules into actively dividing cells during postnatal DNA synthesis. Using this method, Bergmann reported that the rate of adult human cardiomyocyte turnover ranged from 1% annually until the age of 25 years, to 0.45% annually by the age of 75 years. This resulted in an estimate of up to 50% cardiomyocyte turnover over a normal life span.

In “Cardiomyogenesis in the Aging and Failing Human Heart,” Kajstura, Anversa et. al. re-investigate this calculation in collaboration with one of the original Bergmann investigators. They hypothesize that given the requisite balance of cell death, regeneration and hypertrophy required to maintain the observed preservation of cardiac mass over time, true cardiomyocyte renewal rates must be much higher than initially calculated. They point out that by current
estimations, the human heart reaches its adult composition of approximately $8 \times 10^9$
cardiomyocytes by the age of 20 years, with up to a 4.5-fold cellular hypertrophy observed in the
study cohort by this age. This implies that cardiac mass could theoretically be preserved over
time even after 80% cardiomyocyte loss via maximal observed hypertrophy. However
approximately 0.006% of myocytes undergo apoptosis per day, resulting in a natural loss of at
least 2.2% myocytes per year, or much greater than 80% cell loss after the age of 20 over an
average lifespan of 75 years. This results in a net negative cellular balance over an average
lifespan using previously reported cardiomyocyte turnover rates.

The current study was therefore designed to independently quantify cardiomyocyte
proliferation rates in healthy aging as well as in failing human hearts. A total of 19 healthy and
17 failing human hearts were obtained within 24 hours of death, and cells from well-perfused
regions of the left ventricle were digested, purified and sorted. Cell ploidy was documented using
FACS analysis and confirmed by confocal microscopy. Decay-corrected carbon-14
concentrations were measured using accelerator mass spectroscopy. Myocyte, endothelial cell
and fibroblast fractions were determined using immunocytochemistry and spectral analysis. The
main analyses included patterns of polyploidy, average carbon-14-determined cell ages, and the
number of cycling/mitotic myocytes by patient age, in both the healthy aging and failing heart
cohorts.

Using this approach, Kajstura, Anversa et. al. report an approximate 8-fold turnover of
cardiomyocytes and fibroblasts, and a 6-fold turnover of endothelial cells over an average
lifespan. Briefly, their methods revealed the following three major findings: 1) Average
myocyte age in healthy human hearts increased postnatally from a cell age of 8 months during
childhood to cell age 7.9 years between the ages of 33 and 46 years. Average myocyte age
remained relatively constant during adulthood but subsequently progressively decreased to cell age 2.6 years by the ages of 68 to 78 years. This translates into calculated cardiomyocyte turnover rates ranging from 7% to 23% of cells per year (peak turnover before age 20, nadir during adulthood, and subsequent increased cardiomyocyte turnover with aging). 2) Average myocyte age in failing human hearts follows a similar pattern over time as in healthy hearts, but absolute cell ages are significantly lower in failing than in healthy hearts, indicating up to a 2-fold greater cardiomyocyte turnover rate in decompensated heart failure. This elevated cardiomyocyte regeneration rate is measured in the setting of an overall decrease in number of cardiomyocytes per unit volume in heart failure due to fibrosis and cell hypertrophy. 3) An average frequency of 23% multinucleated adult myocytes, the majority of which were binucleated and developed by the age of 25 years. This finding was independent of heart health.

These values indicate a degree of adult cardiomyocyte turnover that is more than an order of magnitude greater than previously reported. Such a high degree of endogenous regenerative capacity would represent an attractive biologic pathway target for therapeutic modulation for cardiac regeneration. Some will argue that the “true” degree of cardiomyocyte turnover may lie somewhere in between the previously reported and current estimations, however Anversa’s current analysis more appropriately satisfies homeostatic equations as described earlier, and the importance of defining biologically plausible rates of endogenous cardiomyocyte repair, as the current study has done, cannot be understated.

There are notable methodologic differences between Anversa’s study in this issue of Circulation and the previous work, which may account for differences in findings. First, Anversa et. al. had access to fresh cardiac tissue which meant that all data needed for carbon-14 analysis could be acquired. In the Bergmann study mathematical modeling was required with the use of
correction factors due to the lack of availability of all data on the preserved specimens.

Differences in calculated cell turnover rates between the two studies are additionally influenced by measure of cell polyploidy rates. Multinucleated or polyploid cells by definition carry greater carbon-14 DNA mass, and may be interpreted as replicating cells, thereby falsely elevating estimations of cell turnover. Estimates of cell turnover are therefore corrected (decreased) in proportion to the frequency of cellular polyploidy measured. In the previously published study, the polyploid fraction was estimated to be 100% in patients above the age of 10 years, rather than the 12% polyploid rate above the age of 25 years directly measured by the gold-standard of FACS by Anversa et. al. Finally, choices in mathematical modeling undoubtedly influence calculated regeneration rates, and the previous study analysis was notable for employing an exponential decay equation to model cell turnover. Such a function was not applied to average cardiomyocyte age measurements by Anversa et. al.

The staggering burden of cardiovascular morbidity and mortality today reflects a threshold of ischemic and hemodynamic injury above which the adult heart exceeds its capacity for endogenous repair. And yet these natural repair mechanisms are quite elegant and robust, relying not only upon adequate diffusion of oxygen and nutrients to at-risk tissue, but also upon dynamic compensatory genetic/molecular signaling and a degree of cellular plasticity maintained throughout adulthood. At what point are these mechanisms overwhelmed? What calculus can we use to predict whether an injury is “too great” to allow favorable remodeling and cellular recovery? Many variables in this equation remain unknown. A clearer understanding of the natural plasticity of myocardium is needed to allow better quantification of injury and anticipated repair, and to guide the strategic development and application of our cellular, paracrine and biomaterials armamentarium. Knowledge of this “natural capacity” for endogenous regeneration
also serves as a benchmark against which the scale and success of our exogenous regenerative therapies can be measured and as a starting point for therapies that seek to leverage or expand this capacity. In this issue of Circulation, Anversa et al. have re-defined the scope of natural repair in the aging and decompensated heart, helping us to better define the calculus of myocardial regeneration. This new understanding of the natural history of myocardial tissue homeostasis will serve us well on the road from scientific understanding to clinical application.

**Conflict of Interest Disclosures:** None.

**References:**


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