After multivariable adjustment for traditional cardiovascular risk factors, race, eGFR, and hsCRP, Aβ40 was independently associated with NT-proBNP, hs-cTnT, left atrial emptying fraction, and Vo_2 max (**Table 1**). Finally, full adjustment for traditional risk factors, renal function, hsCRP, education level, yearly income, and microalbuminuria revealed that Aβ40 remained significantly associated with NT-proBNP and Vo_2 max (**Table 1**). Aβ40 also correlated with a high CAC score, but significance was lost after adjusting for age and eGFR (**Table 1**).

In summary, plasma Aβ40 is associated with a cumulative risk factor profile, with aging, renal dysfunction, non-black race, and the level of triglycerides being the major independent determinants of its variability in the general population without clinically overt CVD. Most importantly, we report here that Aβ40 is associated with subclinical cardiac disease as evidenced by the positive association with the cardiac stress and injury markers NT-proBNP and hs-cTnT, irrespective of traditional cardiovascular risk factors, renal function, and systemic inflammation. Increased circulating NT-proBNP is indicative of increased LV stretch and filling pressures, whereas increased hs-TnT may reflect subclinical myocardial damage in subjects without overt coronary heart disease (4). Interestingly, Aβ40 remained associated with NT-proBNP and Vo_2 max even after considering an additional multivariable model with full demographic characteristics of the population, arterial blood pressure, blood lipid profile, and urine microalbumin levels as a marker of early renal and vascular dysfunction. Given that the cytotoxic Aβ40 peptide accumulates in heart tissues (5) and is independently associated with lower cardiorespiratory fitness in our study, our findings support the notion that plasma Aβ40 may reflect both cardiovascular aging and health status in general population. Further prospective studies are warranted to evaluate the prognostic value of plasma Aβ40 levels for the development of CVD and cardiovascular events in the general population.

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Human Cardiac Fibroblasts Engage the Sarcoplasmic Reticulum in Induced Pluripotent Stem Cell-Derived Cardiomyocyte Excitation–Contraction Coupling

Ca^{2+}-induced Ca^{2+} release (CICR), the process whereby a small influx of Ca^{2+} across the sarcolemma triggers a significantly larger Ca^{2+} release from the sarcoplasmic reticulum (SR), is at the heart of efficient cardiac excitation-contraction coupling (ECC). This process is rudimentary in neonatal cardiomyocytes and significantly diminished in many forms of cardiac disease (1). As such, SR regulation has long been a potential therapeutic target of great interest.

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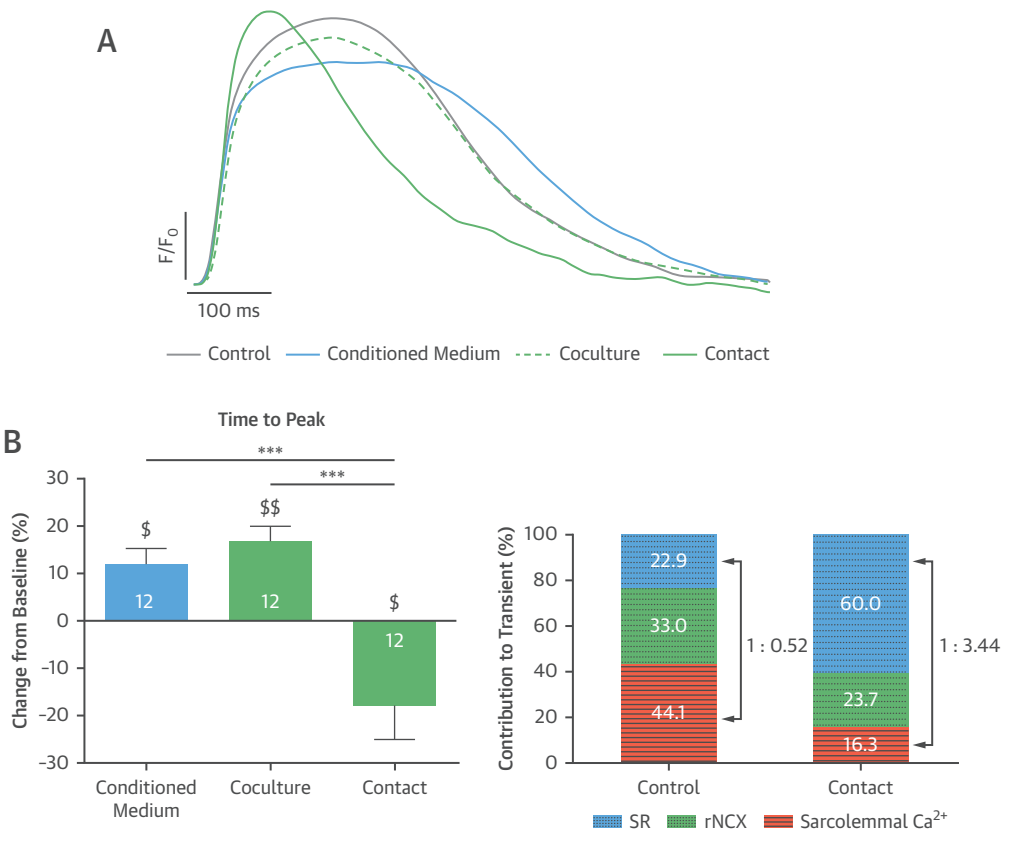
FIGURE 1 Effects of Cardiac Fibroblasts on iPSC-CM Ca^{2+} Transients



(A) Representative Ca^{2+} transients from the 3 culture conditions: fibroblast conditioned medium, coculture (bidirectional paracrine), and direct cell-cell contact. **(B)** Effect of fibroblasts on Ca^{2+} transient time to peak, and percentage contribution to the Ca^{2+} transients, assessed by caffeine application. Values are mean ± SEM. n = number of preparations. ***$p < 0.001$, while $$p < 0.05$, and $$$p < 0.01$ versus control. iPSC-CMs = induced pluripotent stem cell-derived cardiomyocytes; SR = sarcoplasmic reticulum.

There is a growing appreciation of the role of the multicellular myocardial syncytium in regulating cardiomyocyte ECC (2). Fibroblasts represent a significant cellular niche in the myocardium, fundamental everywhere from cardiac morphogenesis to the response to myocardial injury. Importantly, fibroblasts have been shown to alter cardiomyocyte ECC (2); however, whether they have a role in regulating cardiomyocyte SR Ca^{2+} cycling is unknown.

To investigate the role of fibroblasts in this context, we utilized the naïve phenotype of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), whose Ca^{2+} handling properties are comparable to neonatal cardiomyocytes (3).

We cultured fibroblasts from failing human hearts with iPSC-CM monolayers (Cellular Dynamics International, Madison, Wisconsin) at a ratio of 2:1, in 3 conditions designed to mimic the potential modalities of fibroblast-myocyte interaction. First, we used conditioned medium generated by fibroblasts for 24 h, allowing 1-way paracrine signaling. Second, iPSC-CMs and fibroblasts were cocultured together but separated by a semipermeable membrane (Transwells, Corning, Corning, New York), allowing bidirectional paracrine signaling. Last, fibroblasts were seeded on top of iPSC-CM monolayers, allowing direct cell-cell contact. After 24 h of culture, iPSC-CMs were field stimulated at 1 Hz, and Ca^{2+} transients were assessed using Fluo-4 AM. n = the number of preparations unless specified otherwise.

Whereas conditioned medium and coculture had no effect on Ca^{2+} decline (time to 80% decay), this was significantly faster in contact culture (−8.39 ± 2.23%, n = 12; $p < 0.05$ vs. baseline). We then

assessed the components of Ca^{2+} removal through caffeine application, as originally described by Bassani et al. (4). Transient decay rate (K) was significantly greater in contact versus control (3.41 ± 0.15 s^{-1} vs. 2.69 ± 0.07 s^{-1}, n = 26:23 cells; p < 0.001), attributable to 4-fold greater SR Ca^{2+} uptake (2.67 ± 0.12 s^{-1} vs. 0.63 ± 0.67 s^{-1} in control, n = 26:23 cells; p < 0.001). Western blotting demonstrated increased SERCA2a expression (1.02 ± 0.08 vs. 0.55 ± 0.01 in control, n = 4; p < 0.01). Contact culture increased the SR to sodium-calcium exchanger (NCX) ratio from 23:70 in control to 60:32, much closer to the ~70:30 observed in adult cardiomyocytes (4).

Contact culture significantly reduced time to transient peak, a surrogate measure of the rate of SR Ca^{2+} release (**Figure 1**). Caffeine-induced transient amplitude, estimating SR Ca^{2+} content, was significantly greater in contact culture compared with all others, with fractional release, the proportion utilized in voltage-triggered Ca^{2+} transients, greater in contact versus control (16.5 ± 0.40% to 37.4 ± 1.00%, n = 35:32 cells; p < 0.001).

Reverse mode NCX (rNCX) may additionally contribute to the Ca^{2+} transient. Application of ORM-10103, a selective NCX inhibitor (5), resulted in a smaller reduction in transient amplitude in contact versus control (−23.7 ± 1.85% vs. −33.0 ± 1.50%, n = 4; p < 0.001), signifying reduced rNCX contribution.

For cardiomyocytes to maintain homeostasis, SR release must equal SR uptake. Thus, percentage contribution of the SR to all transient Ca^{2+} is equivalent to the percentage uptake calculated previously. Sarcolemmal Ca^{2+}, such as that entering via L-type Ca^{2+} channels, constitutes the remainder after accounting for rNCX. The sarcolemmal to SR flux ratio, a measure of CICR efficiency, was increased from 1:0.5 to 1:3.45 by contact culture.

Overall, our data establish a role for cardiac fibroblasts in regulating SR Ca^{2+} cycling, demonstrating increased SR functionality and engagement in the process of ECC through a mechanism requiring contact or proximity. Future studies will determine whether these effects are brought about through local paracrine signaling or some component of direct cell-cell interaction. Given the significance of diminished SR function and CICR efficiency in cardiac disease, these findings provide valuable insight into the regulation of this fundamental component of cardiomyocyte function.

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In-Scaffold Neoatherosclerosis

An Overestimated Entity?

We read with interest the paper by Moriyama et al. (1) that was published in the *Journal*. The authors investigated the serial neointimal tissue changes in terms of neoatherosclerosis between 1 and 5 years after bioresorbable scaffold implantation as assessed by optical coherence tomography. The reported incidence of neoatherosclerosis was 100% at 5 years.

A therapeutically relevant possibility not taken into account in this study is that the underlying atherosclerotic plaque progression might have as well led to the observed findings (2). If this holds true, the concept that bioresorbable scaffolds seal atherosclerotic plaques would be challenged. Because scaffold struts are no longer visible at 5 years, the authors have arbitrarily assumed a neointimal thickness of 200 μm to distinguish between neoatherosclerosis and underlying native plaque progression.