Fabrication and investigation of cardiac patch embedded with gold nanowires for improved myocardial infarction therapeutics

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ABSTRACT

In the current study, we fabricated a cardiac patch composed of gold nanowires incorporated in collagen fibres by electrospinning followed by chemical crosslinking. Two other counterparts of the cardiac patch were fabricated; one with gold nanowires incorporated in collagen fibres without chemical crosslinking and other with only collagen fibres. The surface morphology of the cardiac patch was viewed via microscopy images and a complete analysis of the mechanical strength of the cardiac patch was done. Swelling and suture resistance tests along with cytotoxicity investigations were performed. Cell adhesion and proliferation were observed on the cardiac patch by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and scanning electron microscope with impediametric studies. This cardiac patch had enhanced mechanical properties and increased hydrophilicity. The cardiomyocytes when seeded this cardiac patch exhibited good cell preservation, growth and sustainability. A layer of cells formed over the cardiac patch was seen by microscopic images because of better cell sustainability. It was observed that in cardiac patch with only porous matrix of collagen fibres, the mechanical strength, suture resistance and cell proliferation were less than when embedded with gold nanowires. Hence, this study clearly exhibited a bioengineered cardiac patch with improved efficacy for management of cardiac infarction.

ARTICLE HISTORY

Received 12 November 2020
Accepted 17 March 2021

KEYWORDS
Cardiac; myocardial; adhesion; collagen; nanowires

1. Introduction

An important reason of mortality along with morbidity throughout the world is failure of the heart to function following myocardial infarction. This is only next to cancer and thrombosis in terms of mortality globally [1, 2]. The weakening out of the infarcted region of the heart followed by development of scar tissue may be one the chief reasons of the failure of the heart [1]. However, the alarming fact is the steeply increased number of patients with heart failure over the recent years [3]. This may be associated with the...
overload of work of the cardiac tissue along with the lining blood vessels. The first approach of treatment is the β-receptor blockers which control the unusual activity of β-receptors present in the membrane of cardiomyocytes thereby contracting the cardiac muscle and blood vessels which in turn reduces work overload of heart [4]. Surgical involvement remains the second approach for treatment using innate or artificial grafts determined by the amount of injury to heart [5]. The advent in bioelectronics have led to the development of stable implant devices which may be helpful in correcting few issues like arrhythmias arising after acute myocardial infarction. However, they are not without limitations. These conventional therapies may be useful in treatment of the affected cardiac muscle and prevent them from further degeneration but cannot repair or rejuvenate the cardiac tissue. Herein lies the utility of nanobio tissue engineered cardiac patches which have created a revolution at the laboratory level in cardiac tissue regeneration. A massive number of vascular and cardiac implants have been created from the bioresorbable/biocompatible polymers like poly-lactic acid (PLLA), poly-lactic-co-glycolic acid (PLGA), and poly-tyrosine-derived polycarbonates [6]. Although these biomaterials have provided a matrix for cell growth they also have been reported for poor cellular adhesion and inferior cellular architecture [7–11]. Nonetheless, few current researches have demonstrated that conjugation of nano bio-materials with these bioresorbable polymers may address the problems [12–13]. But nano biomaterials have also been associated with increased cytotoxicity. Therefore, the solution lies in fabrication of a novel cardiac patch with nanomaterial having minimised cytotoxicity and electrically conductive properties embedded in some natural polymer.

Therefore, we have constructed a novel cardiac patch with the extracellular matrix made up of gold nanowires embedded in collagen. Collagen is natural component for adhesion of cells as well as is the major load-bearing system of the entire skeletal system. It is generally identified as an ingrained framework of body rather than foreign invasion. Moreover, being a natural substance, it has almost no cytotoxicity [14–16]. Furthermore, the chief benefits associated with gold nanomaterials are their improved electrical conductivity, cytocompatibility, novel microarchitecture and easy modification [8, 17–19]. There have been studies reporting hydrogel synthesised of alginate embedded with gold nanowires to form a cardiac patch but alginate was unfortunately found to be unsuitable for cellular growth and development [20].

We designed the study to fabricate cardiac patch utilising electrospun method followed by chemical crosslinking procedures. The cardiac patches fabricated by both the processes were intended to repair the damages caused by myocardial infarction. A bioengineered cardiac patch should be able to help healthy cardiomyocytes grow at the area of infarction and finally disintegrate. Our cardiac patch consisted of core which was made up of gold nanowires which were made to cut & suture and improve the tensile strength for functioning in the wall of ventricles. Surrounding the core was collagen fibres which would help adherence of cardiac cells and their growth. The surface of fabricated cardiac patches was assessed by electron microscopic analysis, and tensile mechanical properties were evaluated. In order to act as cardiac patch for therapeutic properties after myocardial infarction, a very high tensile strength would be needed. Subsequently, the adherence of the gold nanowire core and collagen was tested followed by measurement of suture strength and the cardiac patches prepared by both the procedures were compared. Thereafter, cardiomyocytes cultured from neonatal rats were utilised to study cellular adhesion along with their morphology. A combination of procedures for preparation of cardiac patch would definitely in aid in a better cardiac patch for further investigations.
This was a preliminary laboratory research to design and investigate a cardiac patch for its mechanical strength along with adhesion properties.

2. Materials and methods

2.1. Synthesis of gold nanowires

The chemicals were procured from Sigma Aldrich, China and were used as instructed from manufacturers unless mentioned otherwise. Gold nanowires were prepared following the procedure of earlier studies [20–22] with adaptive changes. 25 ml of 0.25 mM of HAuCl₄ and sodium citrate was prepared to which 0.6 mL of 0.1 M ice-cold aqueous NaBH₄ solution was added with non-stop rigorous stirring (1200 rpm using mechanical homogenizer). This aqueous solution became deep red indicating the formation of colloidal gold. The solution was allowed to stand for 24 h at room temperature. The gold colloid stored at 4°C would be stable for next few months. This is termed as seed solution. Nanowires were formed in three steps. A growth solution was prepared by mixing 8 grams of 0.1 M CTAB with 200 ml of deionised water at physiological temperature at 37°C. As soon as CTAB was completely dissolved, 100 ml of 0.25 mM of HAuCl₄ was mixed along with 650 ml of 0.5 mM ascorbic acid. The solution changed colour from dark to light yellow under the influence of ascorbic acid, due to reduced Au (III) to Au (I). Finally, 100 µL 70 mM of HNO₃ addition was done. This was termed as the core growth solution.

For the initiation of nanowire formation, the core growth solution was divided amongst 3 flasks: Flask 1 (9 ml), Flask 2 (18 ml) and Flask 3(170 ml). Flask 1 and 2 were Erlenmeyer flask and Flask 3 was round bottom flask.

With rigorous stirring, 1 mL seed solution was added to Flask 1. 1 mL solution was added from Flask 1 to Flask 2 after 15 s along with rigorous stirring. Yet again transferring of 5 ml of solution was done from Flask 2 to Flask 3 after 30 s following rigorous stirring. Within Flask 3, the solution was sustained at 37°C with continuous stirring and the colour changed to deep purple over 2 h. The solution resulting was then subsequently purified and kept in centrifuge tubes of capacity 50 mL which were further placed in 37°C oven uninterrupted for about a week. Brown pellets were formed containing approximately 85% wires at the bottom of each tube. The supernatant was rejected and resuspension of the pellets were done in deionised water.

2.2. Preparation of the collagen embedded gold nanowires patch by electrospun method followed by cross linking

The collagen-gold nanowire patch was prepared following the steps described by [20, 23]. The cardiac patch was prepared from collagen obtained from bovine Achilles tendon from Sigma Aldrich, China. Collagen was converted to fibre collagen by inoculation with Staphylococcus aureus (ATCC 29213). Aseptic environments were sustained to circumvent contamination. The process was adapted from [14] with numerous changes. The obtained collagen fibres were kept in dust free environment. The collagen fibres dispersed in demineralised water (1% w/v) were rapidly mixed with gold nanowire solution (1 mg/ml). Thereafter the collagen/gold nanowire solution was chemically cross-linked by adding calcium gluconate following which they were freezeed at −20°C. They were then lyophilised to produce cardiac patch which were of size 3 mm × 2 mm. Some cardiac patches were also made by only rapid mixing of collagen fibres with gold nanowire solution without
crosslinking. Only collagen fibre cardiac patches were produced without adding gold nanowire solution but following the rest of steps. The collagen/gold nanowire cardiac patch with chemical crosslinking would henceforth be termed as COL-AuNW-CP1, collagen/gold nanowire cardiac patch w/o chemical crosslinking would be COL-AuNW-CP2 and only collagen fibre cardiac patch will be known as COL-CP.

2.3. Morphology of the cardiac patch

Gold nanowires, COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP were dehydrated in graded ethanol series and fixed on aluminum stubs by applying double sided tapes. They were covered with gold layer of 20–30 nm by gold sputtering coating unit. Analyses of the samples were carried out using JEOL-JSM scanning electron microscope in a range of 12–20 kV for scanning electron microscopy (SEM). Elemental analysis via SEM was also done to confirm the presence of gold.

For transmission electron microscopy (TEM), 2 mg/ml samples were prepared in Milli-Q water at pH 3.0 and were coated on copper mesh followed by staining with 1% phosphotungstic acid and air-drying. The copper mesh was observed with a JEOL-JEM 100 FX TEM microscope Japan at 90 kV.

2.4. Mechanical testing of the cardiac patch

Tensile testing of the cardiac patch was done using Instron series II automated materials testing system and a procedure elaborated by [24]. The rectangular strips from the cardiac patches were snipped of sizes 30 × 10 mm, and an extension rate of 10 mm/min was realised. Pneumatic grips were utilised to hold the ends of the patch with the grip pressure being 40 Psi. Calculation of tensile strength was carried out along with the Youngs’ Modulus and the stress versus strain curve was plotted.

2.5. Suture tension analysis

The measurement of the final suture tension of the COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP was calculated and was equated with an estimated tension of the left ventricular stitch. The procedure was adapted from [13] with minor changes. Briefly, 20 × 4 mm samples were removed from each cardiac patch, and a with 6–0 suture thread made of polypropylene, a suture of 3 mm was made. Measurements of the ultimate tensile forces on the sutured patches were carried out by uniaxial force testing with aid of a Bose Enduratec ELF3200 (USA). In the left ventricle of human heart, the anticipated maximum stitch tension of any patch embedded would be decided by the following formula:

\[
\text{Tension (in Newton)} = p_w \times \delta_w \times d_s
\]

\(\text{... ... ... ...}\)

Here in the equation, \(p_w\) is the left ventricular end systolic pressure estimated to be 138 ± 20 mm of Hg [25], \(\delta_w\) is left ventricular thickness of the wall estimated to be 13.5 ± 2 mm [26] and \(d_s\) is the estimated distance between sutures fixed at 2 mm.

2.6. Swelling of the cardiac patches

The swelling tests were determined by a procedure elaborated by [27] with few changes. Swelling tests were done on patches of 10 × 10 mm of COL-AuNW-CP1, COL-AuNW-
CP2 and COL-CP utilising a 10 mm side and 5 replicates for each cardiac patch. To begin the tests, first the cardiac patches were desiccated in oven at 37°C for 24 h to expose them to vapours of water at the same temperature. Calculations of swelling ratio for each cardiac patch were done using the formulae:

\[
\text{Swelling Ratio} = \frac{[W_s - W_d]}{W_d} \times 100
\]

Where \(W_s\) is the swollen weight of the cardiac patch and \(W_d\) is the dry weight of the cardiac patch.

Unless the cardiac patches reached a constant weight indicating balance in water uptake, the experiments were continued.

### 2.7. Cytotoxicity of the cardiac patches

The in vitro cytotoxicity investigation was done as per ISO 10993-5:2009 [28]. The MTS assay which is a colorimetric procedure for evaluation of cell viability was performed to evaluate cytotoxicity of cardiac patch [29]. Concisely, the MTS tetrazolium compound (Owen’s reagent) is reduced biologically into a coloured formazan substance by live cells. The formation of the quantity of formazan is directly proportional to the viable cells present in the culture.

#### 2.7.1. Pre-treatment of cardiac patch

Cardiac patches of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP (100 × 100 mm) underwent sterilisation by ethanol (70% aqueous solution) followed by UV light exposure for half an hour. After the procedure, each patch was supplemented with 5 ml of Dulbecco’s Modified Eagle Medium (DMEM). For a time period of 5 days, the patches were kept in water shaker at 37°C. After incubation, the patches were extracted and used for MTS study.

#### 2.7.2. Cell culture

Seeding of the 3T3 cells were derived from mouse fibroblast cell line was carried out onto 96-well plates at of 9 × 103 cells/cm² for an exposure period of 72 h for the MTS assay.

#### 2.7.3. Procedure

Following the seeding of cells for 24 h, the extracted patches were added to each well @ 100 µl per well. They were then gestated for 24-48 h at 37°C in a CO2 environment. For control, DMEM was supplemented to the wells. The medium was substituted with 150 µl MTS (17% solution v/v in DMEM in absence of phenol red) in each well after the incubation time for 24 and 48 h. These were again incubated for 2 h at 37°C in a CO2 environment following which 100 µl aliquots were removed from the wells for relocation to new wells after which at 490 nm the absorbance was noted (ELISA Ultra Microplate Reader USA).

### 2.8. Cell adhesion and proliferation

To observe cell adhesion and multiplication, we have again performed the MTS assay [27]. A similar kind of experiment as mentioned above was performed. Initially, square COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP of sizes 1 × 1 cm were cut and they were sterlised by 70% ethanol followed by UV radiation as mentioned above. Following decontamination, the samples were transferred in 24 well plates within each well followed
by addition of 100 µl of culture medium. The medium for culture was complete and comprised of elevated glucose DMEM comprising sodium pyruvate supplemented with foetal bovine serum (10%), L-glutamine (1%) and penicillin–streptomycin (1%). COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP were allowed to incubate in the well for their hydration in CO2 environment at 37°C for a time period of 2 h.

2.8.1. Cell culture
In culture medium prepared above, rat embryo ventricular cardiomyocytes of the H9C2 cell line were cultured. Upon attainment of confluence, trypsinization of the cells were done following by cell seeding at the density of 5.2 × 10⁴ cells/cm² with COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP.

2.8.2. MTS assay
At a gap of 3 and 7 days after cell seeding, the MTS assay was carried out. The samples were transferred to new wells at the culmination of conventional time interval and washed thrice with PBS. Thereafter, to each cardiac patch, 720 µl of MTS was added (17% solution v/v in DMEM in absence of phenol red). These were again incubated for 2 h at 37°C in a CO₂ environment following which 100 µl aliquots were removed from the wells for relocation to new wells after which at 490 nm the absorbance was noted (ELISA Ultra Microplate Reader USA).

2.8.3. SEM studies
At the end of the experiment on 7th day, the cell samples treated with cardiac patches were analyzed by SEM as described in a process as described above. The samples were gold coated and observed through JEOL-JSM microscope to view the cell growth.

2.9. Impediametric studies
For cell growth and proliferation, impediametric studies were performed too. The ECIS device involved 8 wells and 10 microelectrodes made of gold with 250 µm diameters present in each well (600 µl volumes with the area of substrate being 0.8 cm²) detected the flow of current through the cells. Within a tissue culture incubator, the culture medium prepared above was utilised for incubation of the ECIS devices for 12 h. Rat embryo ventricular cardiomyocytes which had attained confluence were gestated for 72 hrs followed by the addition of following substances: COL-AuNW-CP1 (0.1 ml), COL-AuNW-CP2 (0.1 ml), and COL-CP (0.1 ml) and further incubated for 48 h. Thereafter, measurement of the impedance values was done within a frequency range from 100 Hz to 1 MHz in a logarithmic scale. Consequently, the impedance data was integrated in ZsimpWin (Ver. 3.10) for fit. The equivalent circuit for the study was derived from [30]. RE, RI, represent the solution resistance, charge transfer resistance while CS and QM represented the capacitance of water and interface impedance of cells respectively.

2.9.1. Statistical analysis
Complete dataset of the study was stated as means and standard deviation (mean ± SD) and Origin 8 was used for data processing. One-way analysis of variance (ANOVA) was employed for the calculations. Statistically significant difference was p value <0.05. Impedance studies were carried out thrice to assure reproducibility and the equivalent relative standard deviations (RSD) was used for data representations.
3. Results

3.1. Morphology of the cardiac patches

In the SEM images demonstrated in Figure 1, it was clearly visible that the electrospun gold wires/collagen fibres were not enough for preparation for growth and adhesion of cells (Figure 1A). The gold nanowires in Figure 1E appeared as hairy structures. When the electrospun gold nanowires were further cross-linked with collagen fibres with calcium gluconate resulted in a stable bee-hive kind of structure (Figure 1B). It was also seen that COL-AuNW-CP1 had a much porous structure than COL-AuNW-CP2 (Figure 1C) which had fewer pores. Only COL-CP (Figure 1D) was seen to be soft and porous which may not be sufficient for development of cardiac patch. These results were reinforced by the TEM images seen in Figure 2. The hair like structures of gold nanowires and the elongated rods of collagen fibres were seen clearly in Figure 2E and A respectively. It was also seen from the TEM images (Figure 2B and C) that the gold nanowires were uniformly distributed throughout the collagen fibres in the cardiac patch. The presence of gold was also verified by the elemental analysis seen in Figure 3. The average diameters of the cardiac patches were demonstrated in Table 1.

3.2. Mechanical testing of the cardiac patch

The mechanical strength of the electrospun patch and cross-linked cardiac patches were analysed. The stress-strain behaviour of the COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP were clearly demonstrated in Figure 4. The initial plateau seen in the stress-strain behaviour may be due to the reorientation of the collagen fibres under application of force. Calculation of the Young’s Modulus was grounded on the slant of the stress–strain curve in the linear section which immediately followed the initial plateau. The values were listed in Table 1. As seen from the table, the values of Young’s Modulus were greatly improved after chemical cross linker was added which formed the COL-AuNW-CP1. This
was also noticed in the tensile strength of the samples. Interestingly, the addition of the nanowires into the collagen fibres also increased the strength at break value where COL-AuNW-CP1 exhibited maximum strength at 5.76 ± 0.25 MPa compared to COL-AuNW-CP2 which exhibited 3.12 ± 0.43 MPa while COL-CP exhibited a strength value of only 2.23 ± 0.14 MPa.

3.3. Suture tension analysis

Suture resistance analysis was performed following American National Standard Institute for the Advancement of Medical Instruments 7198, 2016 [31]. The maximum force recorded during the test was a measurement index for suture resistance. The graphs showed in Figure 5 clearly depicted the results of the suture resistance test executed on COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP. Strength of suture resistance was quantified to be 4.6 ± 0.45 N in case of COL-AuNW-CP1 which was 1.6 ± 0.31 N in case of COL-CP and 3.9 ± 0.1 N in case of COL-AuNW-CP2 respectively.

3.4. Swelling tests

Swelling tests of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP elaborated some interesting finds which were depicted in Figure 6. It remained to be seen that whether incorporation of gold nanowires into the collagen fibres caused a change in its swelling characteristics. It was observed that the maximum swelling value of COL-AuNW-CP1, COL-AuNW-CP2 peaked at day 8 after which there was no more swelling. This indicated the beginning of the dissolution of cardiac patches. However, it was interesting to note...
that the peaking of swelling for COL-CP was 6th day instead of 8th day as evident in other two. But the maximum degree of swelling for the COL-CP was 130% which was 105% in COL-AuNW-CP1 and 109% for COL-AuNW-CP2.

3.5. Cytotoxicity tests

In our study, MTS assay was carried out to understand the toxicity of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP. The COL-CP was considered to be the positive control since it was fabricated out of collagen, a natural polymer which had no proven cytotoxicity. The control was supplemented with DMEM. The graphs for cytotoxicity results were collected in Figure 7. It was clearly seen that after overnight gestation with 3T3 fibroblast cells, COL-AuNW-CP1 and COL-AuNW-CP2 on comparison with COL-CP exhibited no significant difference in the absorbance while DMEM supplemented cells exhibited similar absorbance. This clearly rules out any cytotoxic effects of the COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP. However at the end of 48h, there was a

Figure 3. The elemental analysis of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP. Note the presence of Au in COL-AuNW-CP1 and COL-AuNW-CP2.

Table 1. Mechanical tensile properties of the bioengineered cardiac patch.

| Cardiac Patch | Diameter (nm) | Ultimate Strength (MPa) | Strain (%) | Young's Modulus (MPa) |
|---------------|---------------|-------------------------|------------|---------------------|
| COL-AuNW-CP1  | 300 ± 25      | 5.76 ± 0.25             | 40 ± 15    | 70 ± 2              |
| COL-AuNW-CP2  | 225 ± 17      | 3.12 ± 0.43             | 72 ± 13    | 59 ± 1              |
| COL-CP        | 146 ± 23      | 2.23 ± 0.14             | 55 ± 12    | 27 ± 2              |
noteworthy increase in the absorbance ($p < 0.05$) in the COL-AuNW-CP1 and COL-AuNW-CP2 treated cells. This indicated a proliferative action of cells on treated with COL-AuNW-CP1 and COL-AuNW-CP2. But this increase in absorbance was not noteworthy in case of COL-CP.

### 3.6. Cell adhesion and proliferation

A similar kind of study as cytotoxicity was carried out for established and extended time intervals to understand the cell adhesion and proliferation over the bioengineered COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP. It was also performed to study the intracellular interactions with nanostructures embedded in the cardiac patch. The results are depicted in Figure 8. It was seen that similar to the cytotoxicity results, the absorbance of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP were not significantly different. The absorbance on 3rd and 7th day in the cells treated with COL-AuNW-CP1 and COL-AuNW-CP2 exhibited increased absorbance indicating improved proliferative action of

Figure 4. Tensile stress and strain of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP.

Figure 5. Suture resistance force exhibited by COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP. Note the highest exhibited force by COL-AuNW-CP1.
the cells but COL-CP did not exhibit any significantly higher absorbance on 3rd and 7th day. The SEM images obtained were seen in Figure 9. The cardiomyocytes were seen to have completely covered COL-AuNW-CP1 and COL-AuNW-CP2. However, the cells over COL-CP were formed into non-uniform aggregates.

3.7. Impedimetric studies

After the addition of COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP into the inoculations of cells in the device, the impedance values were observed in Figure 10. It was seen that due to the cell proliferation and attachment, there is an increased cellular metabolism which results in lots of by-products which increase the impedance of the solution. Hence,
the impedance values are high but further decreases since at low frequencies, cells do not act as conductors and offer a lot of resistance to the flow of current. This was clearly depicted in Figure 10. It was also noted that the magnitude of impedance decreases increasingly with frequency increase. It is known that at higher frequencies the current permeates the cell instead of moving around the extra-cellular spaces as seen in lower
4. Discussion

The winning strategy that we adopted for the preparation of the cardiac patch was the use of collagen in the patch since extracellular matrices of heart tissues are composed of proteins and polysaccharides. The substances used for making a bioengineered cardiac patch should definitely mimic the original substance. This has been an important aspect in the engineered scaffolds for cardiac tissue engineering in the last decade or so [32–34]. The collagen fibres were integrated with the positively charged CTAB capped gold nanowires to form a functional cardiac patch which was further crosslinked by calcium gluconate. The mechanism of integration of collagen and gold nanowires may be hypothesised as per studies by Tang et al [35]. The collagen molecules underwent a partial unfolding in the helical structure to partially adsorb the gold nanowires. The rapid association of collagen with gold nanowires may be due to their associated positive charge. The gold nanowire provided architecture to the cardiac patch that improves its ease of adhesion to the cardiac tissues. The porous biological matrices synthesised for cellular growth for further organisation in tissues were limited due to their poor conductivity. The embedding of gold nanowires in the patches provided support and most importantly, improved electrical signal transfer between adjacent cells which would help the growth of thicker and better aligned cells than those grown on only collagen matrices. The better and thicker cellular growth was clearly seen in the SEM and TEM images. The gold nanowires mimicked the pore wall of the cardiac patch. Under the optimised conditions, very even and beadless gold nanowires were formed. This has been amply proved in the electron microscopy images. From the images it was verified that there was successful fabrication of artificial cardiac patch mimicking the original tissue. The analysis of the mechanical strength of the electrospun and cross linked cardiac patch revealed an interesting phenomenon. The initial plateau seen in the stress-strain behaviour may be due to the reorientation of the collagen fibres under application of force. The reason for the improved values of Young’s modulus in case of chemically cross linked COL-AuNW-CP1 may be the improved interaction between the collagen fibres and gold nanowires due to the chemical crosslinking due to which the tensile stress may have been transferred to the nanofibres of the cardiac patches. When we compared the uniaxial tensile properties and the young’s Modulus of innate cardiac tissue [36–37], we found that mechanical properties of engineered cardiac patches matched with that of the innate myocardium. However according to these studies it was noted that a little more mechanical stiffness than native myocardium may provide better attenuation of the engineered cardiac patches.

Although there is currently no literature available for suture resistance of myocardium, it has been recorded that human arteries have a suture resistance of 1.96 N [38]. The results of our particular experiments had elaborated that the cardiac patches fabricated with gold nanowires with or without the chemical cross linking have more suture resistance than human arteries. The reason may be hypothesised on a qualitative level that forces that were attributed for detachments at various levels between the fibrous layers were variable but the ones that caused the real breakages in the meshes of the network of fibres were the ones that really contributed to the suture resistance. Therefore a small variability in force may not be actually important. Optimisation of the biomaterials with the tissues remained an important task for development of bioengineered novel scaffolds for aid in nanobioengineering. The swelling capacity and the hydrophilicity of the
biomaterials remain an important parameter in characterisation of the biomaterials. This is essentially due to the fact that the hydrophilicity of the biomaterials influences the cell adhesion itself and swelling influenced the adhesion as well as migration of cells within itself [39]. The decrease in swelling value may be a result of the incorporation and cross-linking of the gold nanowires with the collagen fibres. However this does not affect their functionality much as seen in the further experiments. The degrees of hydrophilicity remain still comparable to native cardiac tissue [40].

It is imperative that these cardiac patches should function inside the body without generating any inflammatory response inside the body. Therefore it was very important to evaluate the cytotoxicity of these fabricated cardiac patches in vitro. Cardiomyocytes were the actual site of action of these patches, hence the cytotoxicity against them were mandatory. But we aimed to observe its cytocompatibility with other normal cells like the fibroblasts. It was vital that they did not incite any cytotoxicity against them. Therefore, the two different cell lines were used in the study. This was vital to be capable of supporting cell adhesion and multiplication in the body. The results of MTS assay indicated an interesting hypothesis. Porous matrix like COL-CP may be cytocompatible but delay cell-cell interaction and electrical signalling which may limit cell growth [41]. But incorporation with gold nanowires may have improved cell-cell interaction for better cellular processes and growth. The sterilised cardiac patches promoted cellular growth instead of inhibiting. There was high absorbance with DMEM treated cells which indicated cell growth and migration. A similar kind of study as cytotoxicity was also performed to understand the intracellular interactions with nanostructures embedded in the cardiac patch. It was also left to be seen whether the gold nanowires which have already been used successfully in cancer therapeutics, delivery and imaging of drugs may augment the cellular excitability. The results indicated that may be the embedded gold nanowires play a significant role in improving the cell-cell interaction by increasing cellular attachment and reproducible branching. This was being hypothesised since COL-CP did not exhibit any significantly higher absorbance on 3rd and 7th day. The SEM images also revealed that the cells do not settle in the pores of the matrices of COL-AuNW-CP1 and COL-AuNW-CP2 but had completely organised themselves all over the surface forming tight aggregated sheet like structure. They were also seen to be integrated in the walls of the pores. Compared to COL-AuNW-CP1 and COL-AuNW-CP2, the cells treated with COL-CP had organised themselves into elliptical aggregates over the matrix which was not uniform. Hence it may be safely concluded that although COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP promote cell growth and proliferation, the proliferation, migration and growth of cells is significantly more with embedded gold nanowires in porous matrix like collagen as compared to only collagen. This improved cardiac patch functionality may be a result of conductive bridges like structures which were formed by the gold nanowires. The conductive bridges like structures were the connections between the cells which may have formed by the gold nanowires through the porous collagen. As may be seen from the TEM and SEM images (1E and 2E), the gold nano wires have hair like extensions which may be the conductive bridges. Furthermore, the improved electroconductivity of the COL-AuNW-CP2 and COL-AuNW-CP1 over COL-CP have been depicted by the impediametric studies. The impediametric studies depicted that due to the cell proliferation and attachment, there is an increased cellular metabolism which results in lots of by-products which increase the impedance of the solution [42]. Hence, the impedance values are high but further decreases since at low frequencies, cells do not act as conductors and offer a lot of resistance to the flow of current. This reinforced our current data where it was proved that cardiomyocytes upon treatment with COL-AuNW-CP1 and COL-AuNW-CP2 along
with COL-CP resulted in increased cell population. However, the increase in impedance of the cells treated with COL-CP is low because of the poor cell interaction and growth owing to the porosity of the matrix.

5. Conclusions and future directions of the work

In the present decade, nanobiomedicine and biomedical engineering emerged as approaches that may maneuver and revolutionise cardiac therapeutics. This has also altered treatment of myocardial infarction by fabrication of naturalised or polymerised cardiac patch. The cardiac patches if were made of conductive nanomaterials may be able to promote cell migration since cardiac cells are electroactive ones. The cardiac patch implanted should assist in ventricular remodelling, dilatation prevention and renewing the infarcted zone. The bioengineered COL-AuNW-CP1, COL-AuNW-CP2 and COL-CP in our study, apart from imitating the extracellular matrix also offered enough mechanical strength to infarcted region to aid in its healing. They possessed enough tenacity to enhance the mechanical properties of disrupted area and aide in cardiomyocytes growth. Hence, their properties included increased mechanical strength, biodegradability and cytocompatibility among others. The COL-AuNW-CP1 was prepared with a chemical cross-linker calcium gluconate whereas no chemical cross-linked was utilised for COL-AuNW-CP2. It was comprehended that while there was no substantial difference between the two in aiding cellular growth and propagation, the mechanical strength of COL-AuNW-CP1 far exceeded that of COL-AuNW-CP2. It was also safely concluded that embedding gold nanowires within collagen matrices provides extra mechanical strength and also improved cell proliferation. It was observed that with only porous matrix like in COL-CP, the mechanical strength, suture resistance and cell proliferation were less than when embedded with gold nanowires. Moreover gold nanowires embedded in collagen matrices may have a definitive role in improving cell-cell interaction by increasing cellular attachment and reproducible branching. The bioengineered cardiac patch did not exhibit any forms of cytotoxicity too. However, this was a preclinical bench study with anticipated results. A further in-detailed study with further cell analysis and in-vivo studies may be undertaken for animal trials.

Acknowledgments

Not Applicable

Disclosure statement

The authors declare no conflict of interest.

Funding

This research received no external funding.

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