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# Biocompatibility evaluation of encapsulated silver-based printed circuits for *in-vitro* long-term sensing devices

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**Abstract**

Metal-based inks, such as silver nanoparticle (AgNPs) dispersions, are standard solutions in printed electronics (PE) due to their high bulk conductivity, stability, and commercial availability. Conductive functional printed patterns have been exploited for several applications, such as (wearable) sensors, antennas, transistors, batteries. Recently, PE has also been widely investigated for biomedical and bioelectronic interfaces. In this case, the choice of biocompatible materials is crucial to ensure biological activities of the seeded cells. For instance, the release of $Ag^+$ ions in the medium culture in *in-vitro* cultures, is known to induce cytotoxicity, in a dose-, size-, and time-dependent way, causing oxidative stress and eventually cellular death. Hence, an encapsulation step of the printed pattern is usually performed to reduce such risk, by inducing however additional limitations and complications on the design and production. This work investigates the biocompatibility of a commercial Aerosol Jet® Printed (AJ®P) AgNPs-based ink ($Ø_{avg,particle\ size}$ = 35 nm, Ag content ~50 wt%) use to print conductive circuits for *in-vitro* bioelectrical sensing. Particularly, the ink was AJ® printed on the top of stereolithography (SLA) samples coated with Parylene-C, and further encapsulated with polydimethylsiloxane (PDMS).
Cell viability assays were evaluated using both in adhesion and suspension cell type, such fibroblasts and B-Lymphoblastoid cell line respectively, at different time points from 7 days till 21 days to verify cellular activity and proliferation for long term usage.
This work provides new insights concerning the use of encapsulated conductive AgNPs-based circuits for *in-vitro* long-term sensing devices.

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*Keywords:* Printing of electronics; Silver nanoparticle inks; Bioelectronics; biocompatibility;

## 1. Introduction

Tissue engineering (TE) is a strongly expanding research area especially connected with the regenerative medicine. Regenerative medicine has been defined as the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage or congenital defects[1]. In particular, great interest is lately focused on the use of stem cells which are in the center of many applications in regenerative medicine because of their extensive ability to self-renew and to generate differentiated progeny. Moreover, stem cells can be easily obtained from a skin biopsy or blood cells of a patient[2-3] in order to create an *in vitro* model of the disease, patient specific useful for drugs screening or pathogenetic investigations[4]. In this context, numerous studies have highlighted the importance of the stem cell microenvironment, or niche, in modulating stem cell behavior[5-6]. *In vivo,* stem cells reside in a dynamic, cell type-specific niche. This

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microenvironment is composed of stem cells, supportive stromal cells and surrounding extracellular matrix (ECM). The niche provides chemical, mechanical, and topographical cues facilitating stem cell renewal and controlling stem cell fate[7].
In order to mimic *in vitro* the natural tissue microenvironment, TE approaches require biocompatible scaffolds, cells, and different applied chemical and physical stimuli.
In this sense, a varying set of bioreactors that properly stimulate those materials and cells *in vitro*, creating an appropriate biomimetic microenvironment, is developed to obtain active bioreactors. Modern bioprocess monitoring demands sensors that provide information about the process state. In particular, sensors for monitoring bioprocesses carried out in single-use bioreactors are needed because disposable systems are becoming increasingly important for biotechnological applications[8-9].
Metal-based inks, such as silver nanoparticle (AgNPs) dispersions, are standard solutions in printed electronics (PE) due to their high bulk conductivity, stability, and commercial availability. Conductive functional printed patterns have been exploited for several applications, such sensors. However, it is known that the choice of the material composition of the sensor is crucial for the health state of the cells in touch with them. Scientific literature teaches us that the release of $Ag^+$ ions in the medium culture in *in-vitro* cultures, induces cytotoxicity, in a dose-, size-, and time-dependent way, causing oxidative stress and eventually cellular death[10]. Hence, an encapsulation step of the printed pattern is needed to reduce such risk, by inducing however additional limitations and complications on the design and production. This work investigates the biocompatibility of a commercial Aerosol Jet® Printing (AJ®P) AgNPs-based ink ($Ø_{avg,particle\ size}$ = 35 nm, Ag content ~50 wt%) use to print conductive circuits for *in-vitro* bioelectrical sensing. Particularly, the ink was AJ® printed on the top of stereolithography (SLA) samples coated with Parylene-C, and further encapsulated with polydimethylsiloxane (PDMS).

## 2. Materials and methods

### *2.1. Substrates fabrication*

SLA samples were coated with the biopolymer Parylene-C (ParC) (~3 µm in thickness), and further treated with a low pressure plasma to enhance substrate wettability. Then, conductive patches (an arc and a line, width = 150 µm) were printed onto the ParC-SLA samples by means of an AJ®P technique, using a water-based AgNPs commercial solution (Novacentrix, Metalon® JS-ADEV N250, USA) (KU Leuven). The ink has an Ag loading content of ~50 wt%, a mean particle size of 50 nm, diethylene glycol (DEG, 3-10 wt%), and isopropanol (IPA, 2-10 wt%) as co-solvents. After a post-sintering process (2 hours at 150 °C), conductive patches were further encapsulated in polydimethylsiloxane (PDMS) in order to avoid a release of Ag+ ions, which are potentially cytotoxic when exposed to the medium culture[10]. Final electrical resistances (R) were detected equal to RArc ~ 11.8 Ω and RLine ~ 5.0 Ω. **Figure 1** reports a representative image of the obtained samples.

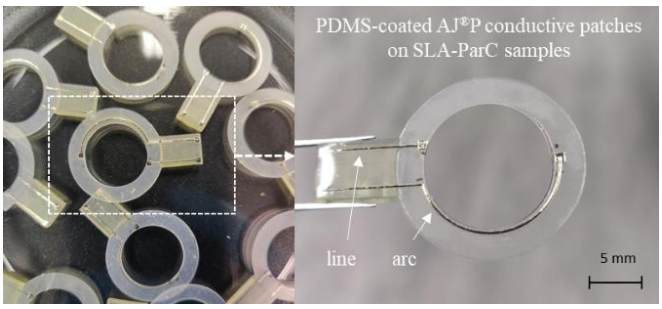


**Figure 1**. Representative image of PDMS-coated AJ®P conductive patches on SLA-ParC samples.

### *2.2. Cell culture*

Human fibroblast (*BJ cell line ATCC® CRL-2522™)* and B-Lymphoblastoid cell line (*HCC1739 BL, CRL-2334™)* were cultured in adhesion in Dulbecco's Modified Eagle Medium (DMEM, Euroclone, Milan, Italy) and in suspension in Roswell Park Memorial Institute (RPMI, Euroclone, Milan, Italy) 1640 respectively, completed with 10% fetal calf serum (FCS, Euroclone), 1% L-glutamine (Euroclone), and 1% penicillin/streptomycin (Euroclone). Cells were maintained at 37 °C, 5% $CO_2$ in a humidified incubator, the medium was changed three times a week, and the cell culture were splitted when a confluence of 70/80% was reached.

### *2.3. Preliminary biocompatibility tests*

Immunofluorescence and cell viability assays were applied to test the cellular adhesion and the biocompatibility on the PDMS-coated AJ®P conductive patches on SLA-ParC substrates. Specifically, immunofluorescence was executed to observe the cellular morphology and adhesion of human fibroblasts (HFs). Cell viability assays were instead executed to evaluate the HFs viability and proliferation. Before cell seeding, all the samples were washed in phosphate-buffered saline solution (PBS) and further sterilized in an autoclave at 121°C for 20 min.

#### *2.3.1 Immunofluorescence with HFs*

Immunofluorescence assay with HFs was performed on PDMS-coated AJ®P conductive patches on SLA-ParC substrates to test the biocompatibility and cellular adhesion (3 replicas). The substrates were inserted into a 6 well plate using a sterilized tweezer. HFs were enzymatically detached from a flask using Trypsin (Euroclone) and washed in order to obtain a single cells suspension that was collected and centrifuged at 1400 rpm for 10 minutes. The supernatant was removed and a concentrated HFs suspension (5 x$10^5$ cells) was deposited onto each support and incubated for 30 minutes before filling each well with a proper volume of complete DMEM. Cells were maintained at 37 °C in a saturated humidity atmosphere containing 95% air and 5% $CO_2$.
In parallel, the same cellular concentration was poured in an empty dish and placed in the incubator at the same conditions, as positive control.

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After 5 days in culture, cells on substrates were fixed using the Fix&Perm Sample Kit® for 30 minutes (15 min fixation and 15 min permeabilization), incubated with blocking solution (iBindTM 5X Buffer-Invitrogen) for 45 minutes, and stained with Phalloidin that mark the cytoskeletal component of the cells. Cell nuclei were then counterstained with 4',6-diamidin-2-fenilindolo (DAPI) for 5 minutes to highlight the cellular nuclei of the living cells. Eventually, the specimens were positioned on glass coverslips on a drop of glycerol and studied by means of an inverted fluorescence microscope (Olympus IX70). Image analysis was performed via the Image-Pro Plus software v.7.0 (Media Cybernetics).

*2.3.2 rATP assay*

To assess the material biocompatibility of PDMS-coated AJ®P conductive patches on SLA-ParC substrates, an rATP assay (CellTiter-Glo® 3D Cell Viability, Promega cat. no. G9681) was executed evaluating the cellular viability and proliferation rate of HFs. In details, an indirect rATP assay was performed at 24, 48, and 120 h (three replicas each), including plastic as positive control. This assay measures the quantity of viable cells by quantifying the adenosine triphosphate (ATP) values present in each well. ATP is recognized as a marker for metabolic cellular activity. PDMS-coated AJ®P conductive patches on SLA-ParC substrates were placed in a 6-well plate and let to release any potential cytotoxic components into 3 mL of complete DMEM or RPMI for 7 days in a humidified incubator at 37°C and in 5% $CO_2$ atmosphere. As reference, the same volume of medium was poured in an empty dish and placed in the incubator at the same conditions. The day before the test, HFs were seeded in flat bottom 48-well plates ($2 \times 10^4$ cell/well). The day after, cell supernatant was removed and replaced by either ink sample conditioned medium or control medium. As experimental positive control cells were also seeded in fresh medium to verify that the medium, maintained at 37°C for 5 days, could not affect the cell healthy. Finally, the cells seeded in the 48- well plates were subjected to an ATP cell viability assay.
The ATP assay was performed according to supplier protocols on triplicated samples. Briefly, a volume of CellTiter-Glo 3D Reagent equal to the volume of cell culture medium present in each well was added to induce cell lysis. After incubation at room temperature for 25 min to stabilize the luminescent signal, the total volume was transferred into a 96-well opaque-walled multiwell plate and the luminescence was recorded using using a Tecan Infinite® M200 microplate reader (Tecan, Männedorf, Switzerland). Afterwards, an ATP standard curve was plotted in the range of 10 µM to 10 nM, using the ribonucleoside triphosphate rATP (Promega cat. no. P1132) as positive control to compare the luminescence values acquired from the standard wells and the and the samples. Hence, the ATP concentration was calculated and plotted.

*2.4. Biocompatibility tests: long term culture*

Cell viability assays were also evaluated using both in adhesion and suspension cell type, such HFs and B-LCLs respectively, at different time points from 7 days till 21 days to verify cellular activity and proliferation for long term usage. The autoclaved substrates were inserted into a 6 well plate using a sterilized tweezer, then HFs and B-LCLs cells were seeded at concentration of 25 x $10^4$ cells/well, three replicas each. As reference, the same cellular concentration was poured in an empty dish and placed in the incubator at the same conditions. Cells were maintained at 37 °C in a saturated humidity atmosphere containing 95% air and 5% $CO_2$ and the medium was changed three times a week. After 7, 14, and 21 days, cells were detached and treated with the Live and Dead staining solution (Abcam, Cambridge, UK) and acquired on a FACS Canto II cytometer (BD Bioscience). Data were collected from at least $1x10^4$ events/sample and analyzed with a FlowJo software (TreeStar, Ashland, OR). Gates of live cells (positive for the FITC channel and negative for the PerCP channel) were setted as per manufacturer's instruction.

*2.5. Statistical analysis*

Data from replicates were expressed as mean ± standard deviation (S.D.). Comparisons between groups were performed by Student's *t*-test, using GraphPad Prism (San Diego, CA).

## 3. Results

*3.1. Cellular adhesion: bright-field and immunofluorescent visualization*

A rapid and extremely preliminary way to investigate the biocompatibility of a material is to culture an adherent cell line, such HFs, on it. In fact, it is known that healthy fibroblasts tend to grow adherent on the surface, spreading their cytoskeletal, while a round floating shape is more related to an unhealthy state. Thus, a concentrated HFs suspension was seeded on PDMS-coated AJ®P conductive patches on SLA-ParC substrates and on plastic dish as positive control. After 5 days in culture were verified the cellular adhesion by an inverted optical microscope (EVOS™ XL) (**Figure 2**) and by immunofluorescence (**Figure 3**). As shown in Figure 2, the HFs were attached on both plastic control and AgNP-based ink substrates.

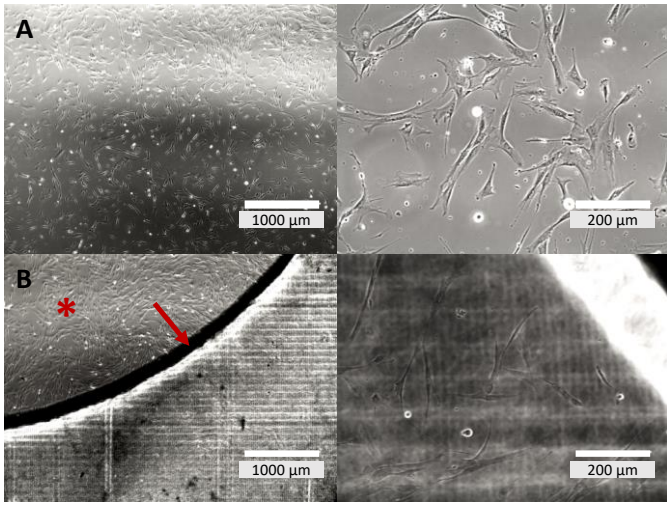


**Figure 2. A)** Representative pictures of HFs cultured for 5 days in complete DMEM on plastic dish as positive control. **B)** Representative pictures of HFs cultured for 5 days in complete DMEM in a dish containing the PDMS-coated AJ®P conductive patches on SLA-ParC substrates.

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With the greater magnification (20X) is clear the spreading shape of HFs characterized by their typical cytoskeletal protrusions, sign of healthy condition. Moreover, from **Figure 2B** is possible to notice the simultaneous presence of HFs attached on plastic well (in the hole of the patches, indicated by the red symbol asterisk) and on the surface of the SLA substrate (indicated by the red arrow). These results suggest that the HFs are able to attached on PDMS-coated AJ®P conductive patches on SLA-ParC substrates, survive in the same medium in contact with the AgNPs-based ink circuit and that the cells adherent on the patches are visible by optical microscope.

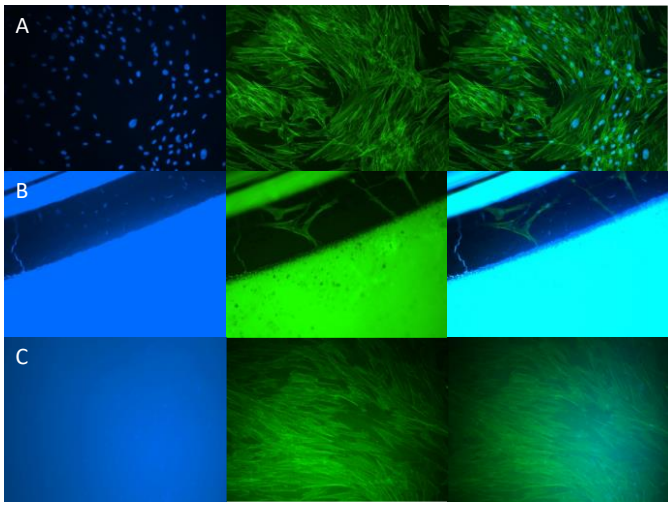


**Figure 3.** Representative pictures of the immunofluorescence performed on HFs cultured for 5 days in complete DMEM on plastic dish as positive control **(A)**, and in a dish containing the PDMS-coated AJ®P conductive patches on SLA-ParC substrates **(B, C)**. In particular, in **B** are shown HFs stained on the AgNPs-based ink circuit, while in **C** HFs stained on the patch's surface. The cytoskeletal marker phalloidin is in green, while nuclei are counterstained in blue. Magnification 10X.

In **Figure 3** is reported the result of the immunofluorescence assay. In particular, is shown the staining of the nuclei (in blue, first column), the staining of the cytoskeletal marker phalloidin (in green, second column), and the merge of both (third column) for HFs adherent on plastic control (A), on AgNPs-based ink circuit (B), and on the patch's surface (C). The staining result confirms the adherence capability of the HFs on both plastic control and substrate. Moreover, from this experiment emerged the strong autofluorescence of the resinous material composing the patch, when excited by blue light, that doesn't allow the visualization of the nuclei (Fig. 3 C). On the substrates, nuclei in DAPI are visible only on the black AgNPs-based ink circuit, probably because the darker ink covers the surface and reduced the emission of the autofluorescence.

### *3.2. Biocompatibility: rATP cell viability assay*

Since the choice of biocompatible materials is crucial to guarantee biological activities of the seeded cells, it is important to verify that the PDMS encapsulation of the AgNPs-based ink ensures not only the cellular surviving, but also allows its metabolic activity. Cells are very sensitive to the environmental stimuli, and if they do not perceive favorable conditions to replicate, arrest their cell cycle into a quiescent phase, while remaining viable. ATP cell viability assay is an enzymatic assay that detects the ATP produced by the cells to convert luciferin into oxyluciferin, which generates a light signal. Thus, the signal collected is a measure of the number of metabolically active cells in culture. A decreased luminescence can be caused by either cell death or metabolic quiescence.
**Figure 4** shows the result of the rATP assay at three times point (24, 48, 120h) performed on HFs cultured in conditioned medium (medium collected from dishes containing a PDMS-coated AJ®P conductive patches on SLA-ParC substrate for 7 days), in control medium (medium poured in an empty dish and placed in the incubator at the same conditions), and in fresh medium, as experimental positive control.

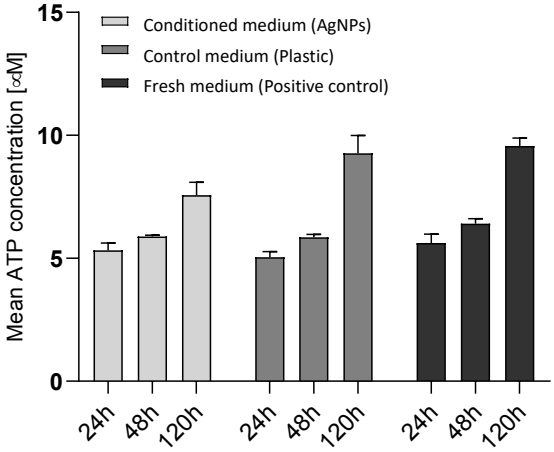


**Figure 4.** ATP concentration measurement in HFs cultured for 24, 48, and 120h either in fresh complete DMEM as positive control, in control medium collected from culture on plastic dish, and in DMEM conditioned for 7 days with PDMS-coated AJ®P conductive patches on SLA-ParC substrates. The graph shows the mean ± S.D. ($n$ = 3).

A positive growth trend of proliferation was observed for HFs in all the three conditions, with no significant differences among them. The similar trend observed between cells cultured in fresh medium and cells grown in control medium, assures that the complete DMEM, maintained at 37°C for 5 days, could not affect the cell healthy. After 120h a moderate, not significant, reduction of ATP concentration detected from HFs cultured in conditionate medium emerged, attributable to an increased cell death or to a slowing of metabolic activity for due to unfavorable environmental conditions.

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### 3.3. Biocompatibility in long term culture: live/dead assay

Since the encapsulated AgNPs-based printed circuits were designed for *in-vitro* long-term sensing devices, it is important to verify their biocompatibility also after a long period of cellular culture. Thus, a live and dead cell assay was performed using both in adhesion and suspension cell type, maintained in culture with the PDMS-coated AJ®P conductive patches on SLA-ParC substrates or in an empty plastic dish, at different time points from 7 days till 21 days. **Figures 5** and **6** describe the result obtained for HFs and B-LCLs, respectively.

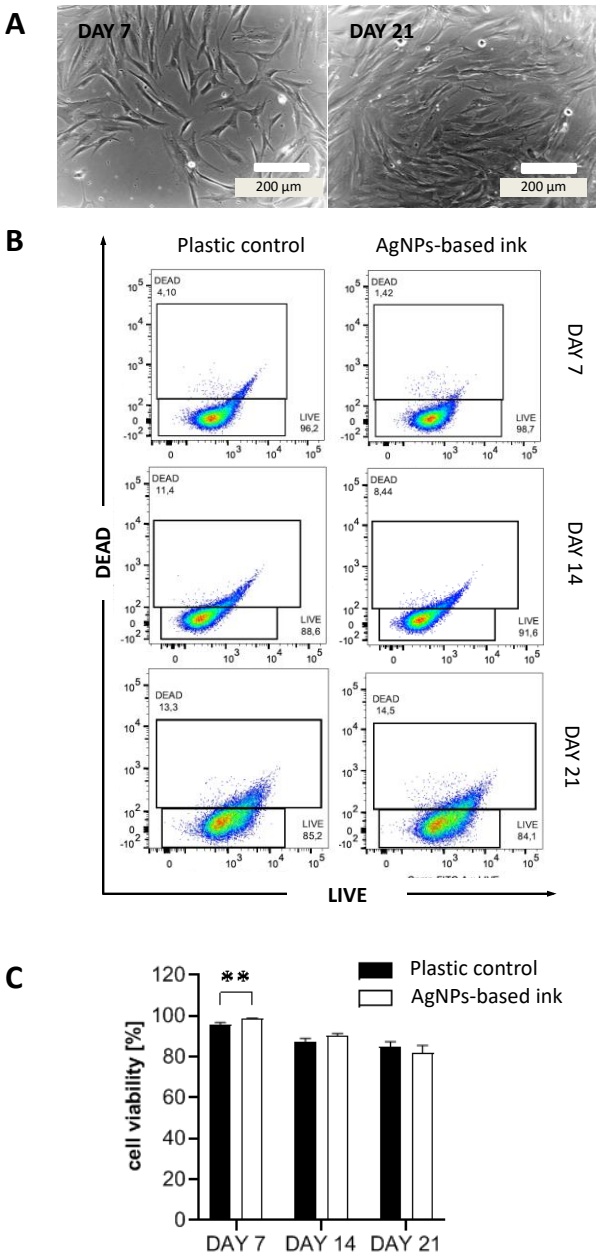


**Figure 5.** HFs cell viability after 7, 14, and 21 days of culture either in complete DMEM on a plastic dish as positive control, and on a dish containing the PDMS-coated AJ®P conductive patches on SLA-ParC substrates. Representative images of HFs after 7 and 21 days of culture **(A)** Representative dot plots of the live/dead cell assay **(B)**. Graph showing the percentage of cell viability as the mean ± S.D. ($n$ =3, ** $p$ > 0.01)

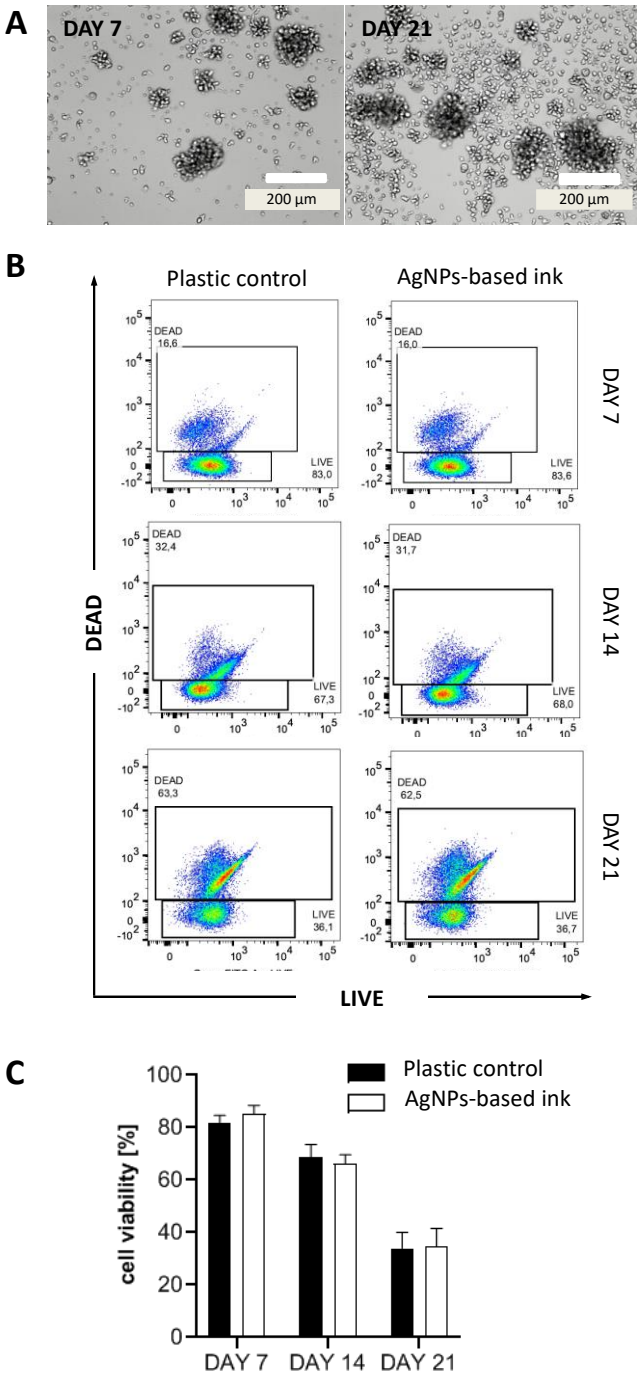


**Figure 6.** B-LCLs cell viability after 7, 14, and 21 days of culture either in complete DMEM on a plastic dish as positive control, and on a dish containing the PDMS-coated AJ®P conductive patches on SLA-ParC substrates. Representative images of HFs after 7 and 21 days of culture **(A)** Representative dot plots of the live/dead cell assay **(B)**. Graph showing the percentage of cell viability as the mean ± S.D. ($n$ =3).

As shown, the PDMS encapsulation seems to ensure the cellular viability even after 21 days of culture for both the cellular typologies. The percentage of cell viability was in fact very similar between PDMS-coated AJ®P conductive patches on SLA-ParC substrates and plastic control. A statistically significant difference between treated and control HFs appeared only after 7 days. However, this result should not

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cause concern as the difference between treated and not treated, is present but minimal, and is statistically significant only given by the very low standard deviation, an indication of a high reproducibility of the triplicate. Finally, the reduced percentage of cell viability measured after 21 days in the B-LCLs culture, similar in both PDMS-coated AJ®P conductive patches on SLA-ParC substrates and plastic control, is probably due to a physiological cell death for over confluence.

## 4. Conclusions

This work concerns the potential biocompatibility of a commercial Aerosol Jet® Printed (AJ®P) AgNPs-based ink (Øavg,particle size = 35 nm, Ag content ~50 wt%) use to print conductive circuits for in-vitro bioelectrical sensing. Particularly, the ink was AJ® printed on the top of stereolithography (SLA) samples coated with Parylene-C, and further encapsulated with polydimethylsiloxane (PDMS).

The PDMS encapsulation step of the printed circuit was needed since is known in literature that the release of $Ag^+$ ions in the medium culture can induce cytotoxicity, in a dose-, size-, and time-dependent way. In order to verify if the encapsulation step was sufficient to avoid the potential toxic release of the $Ag^+$ ions, a deep biocompatibility investigation was performed.

Bright - field and immunofluorescence images of human fibroblasts cultured in adhesion on PDMS-coated AJ®P conductive patches on SLA-ParC substrates attested the preliminary results of biocompatibility. In fact, it is known that healthy fibroblasts tend to grow adherent on the surface, spreading their cytoskeletal, while a round floating shape is more related to an unhealthy state. Moreover, since a biocompatible material should also guarantee the biological activities of the seeded cells, it is important to verify that the PDMS encapsulation of the AgNPs-based ink ensure not only the cellular surviving, but allow also their metabolic activity. For this reason, we analyzed the rATP concentration, recognized as a marker for metabolic cellular activity, of fibroblasts cultured in conditioned medium with substrates for 7 days. A positive growth trend of proliferation was observed for HFs cultured in conditioned medium and in control medium with no significant differences among them.

Finally, since the encapsulated AgNPs-based printed circuits were designed for *in vitro* long-term sensing devices, the biocompatibility after a long period of cellular culture was evaluated. In particular, a live and dead cell assay was performed using in adhesion (HFs) and suspension (B-LCLs) cell type, maintained in culture with the PDMS-coated AJ®P conductive patches on SLA-ParC substrates or in an empty plastic dish, for 21 days. The percentage of cell viability analyzed after 7, 14 and 21 days in culture was very similar between PDMS-coated AJ®P conductive patches on SLA-ParC substrates and plastic control. For these reasons, we can conclude that PDMS encapsulation seems to ensure the cellular viability and proliferation, even after three weeks of culture for both adherent and suspended cellular typologies. Further cell viability assays to test the performance of the PDMS-coated AJ®P conductive patches on SLA-ParC samples in a fluid dynamic of a bioreactor will be performed.

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## References

1) Castro N., et al.; Physically Active Bioreactors for Tissue Engineering Applications. Advanced Biosystem. 2020, Vol 4 issue 10
2) Ali E, et al. Generation of induced pluripotent stem cell (iPSC) lines from a Joubert syndrome patient with compound heterozygous mutations in C5orf42 gene. Stem Cell Research 2020;49:102007
3) Lanzi G. et al.; Generation of 3 clones of induced pluripotent stem cells (iPSCs) from a patient affected by Autosomal Recessive Osteopetrosis due to mutations in TCIRG1 gene. Stem Cell Research, 2020, 42, 1016604
4) Genova E., et al.; Biomarkers and Precision Therapy for Primary Immunodeficiencies: An In Vitro Study Based on Induced Pluripotent Stem Cells From Patients. Clinical Pharmacology and Therapeuticsthis, 2020, 108(2), pp. 358–367
5) Ginestra P., et al..; Selective laser melting and electron beam melting of Ti6Al4V for orthopedic applications: A comparative study on the applied building direction. Materials, 2020, 13(23), pp. 1–23, 5584
6) Ferraro RM. et al.; Carbonization of polymer precursors substrates to direct human iPSC-derived neurons differentiation and maturation. Procedia CIRP, 2020, 89, pp. 39–44
7) Lane S et al.; Modulating the stem cell niche for tissue regeneration. Nature Biotechnology 2014; 32: 795 803
8) Busse C., et al.; Sensors for disposable bioreactors. England Life Science 2017, 17, 940–952
9) Bisgaard J., et al., Flow-following sensor devices: A tool for bridging data and model predictions in large-scale fermentations. Computational and Structural Biotechnology Journal. Volume 18, 2020, Pages 2908-2919
10) Seiti M., et al.; Nebulized jet-based printing of bio-electrical scaffolds for neural tissue engineering: a feasibility study. Biofabrication, 2020, 12(2), 025024

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