

VIII Jornades IdISBa

PÒSTERS DE L'ÀREA: Transversal de Ciència i Tecnologia en Salut



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Lab-on-Chip VOCs sensing: Acetone detection in exhaled human breath as non-invasive diagnosis tool for diabetic patients

Non-invasive diagnosis: Diabetes

Detection goal: sub-ppm acetone



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Biosensors, Instrumentació Mèdica i Anàlisi de Dades (E-BIOSENS)

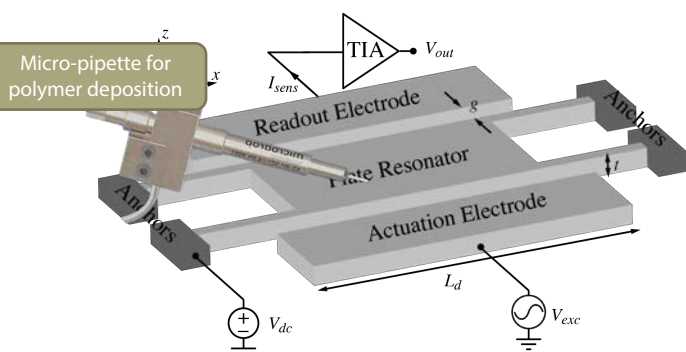


Summary

With studies reporting over 870 [1], Volatile Organic Compounds (VOCs) present in human exhaled breath are promising candidates to mirror metabolic processes for non-invasive diagnosis [2]. VOCs are present with concentrations ranging from parts-per-trillion (ppt) to parts-per-million (ppm) [3]. As a diabetes biomarker, acetone levels of 300 ppb have been demonstrated in healthy individuals [4], while increasing up to 1.8 ppm in diabetic patients [5]. However, available commercial equipment do not reach such resolution or present expensive and bulky solutions.

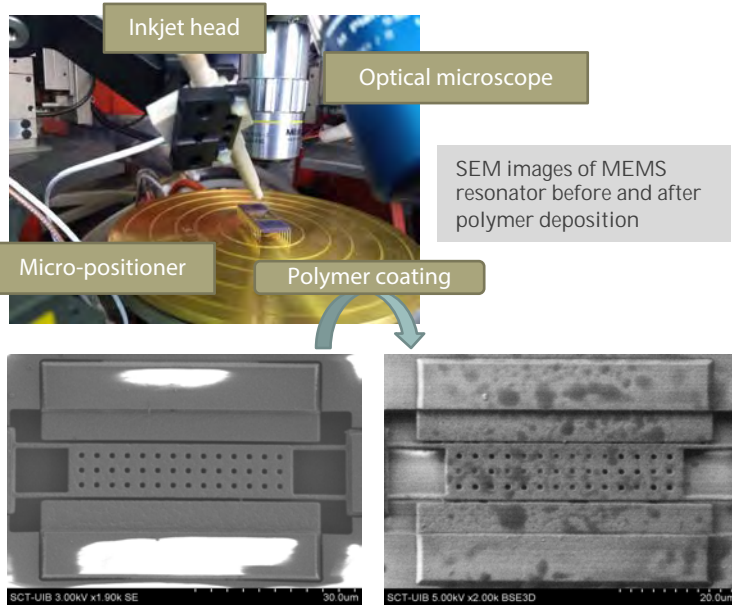
Thanks to the continuous advances in microelectronics technologies and miniaturization capabilities, a new generation of sensing systems has emerged following a Lab-on-Chip (LoC) configuration. They integrate monolithically a gas sensing element (MEMS resonator based), together with the readout and communication circuit (CMOS) in a single chip. Thus, offering high portability, low cost, outstanding limit of detection (LOD) and point-of-care diagnosis [6].

MEMS resonators are mechanical elements at the micro-scale whose resonance frequency changes due to a deposited mass, operating as a mass sensor. Using a MEMS resonator coated with a specific functionalization polymer (P4V) layer that can capture the target molecule, this work presents a LoC VOCs sensor for acetone detection down to 20 ppb [7].



MEMS resonator schematic with integrated circuit (TIA). SEM image.

Polymer Coating: Inkjet Deposition



References

- [1] Costello, B.d.L. et al. Jour. of Breath Research. 2014, 8, 1, pp. 014001.
- [2] Sethi, S. et al. Cll. Microbio. Rev. 2013, 26, 3, pp. 462-75.
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- [4] Alizadeh, N. et al. IEEE Sensors Journal. 2020, 20, 1, pp. 5-31.
- [5] Tripathi, K. et al. Carbon. 2016, 110, pp. 97-129.
- [6] Li, S.S. In IEEE-NANO. 2016, pp. 456-9.
- [7] Perelló-Roig, R. et al. Lab on a Chip. 2021, 21, pp. 3307-15.

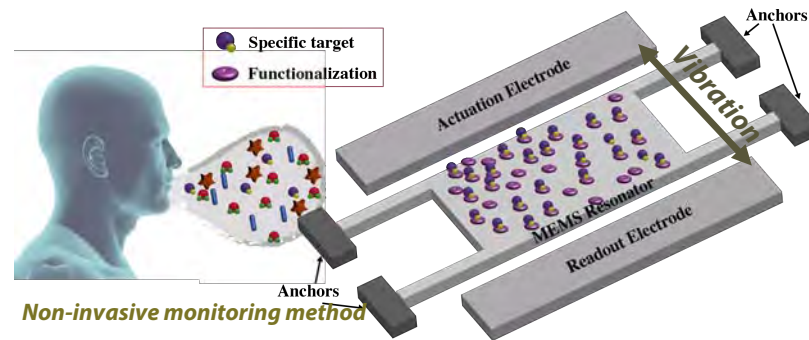
Acknowledgements

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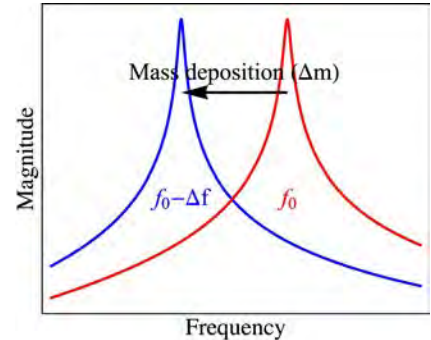


Principle of Operation



Non-invasive monitoring method

Functionalization layer captures acetone molecules on MEMS resonator



Resonance frequency decreases due to the deposited mass

Stiffness constant: k

Resonator mass: m

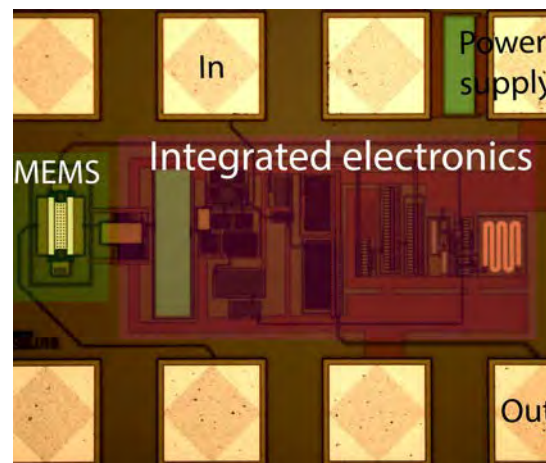
Natural frequency: f_0

Frequency change: Δf

$$f_0 = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \Rightarrow f_0 - \Delta f = \frac{1}{2\pi} \sqrt{\frac{k}{m + \Delta m}} \Rightarrow \Delta f \approx \frac{1}{2} \frac{\Delta m}{m} f_0$$

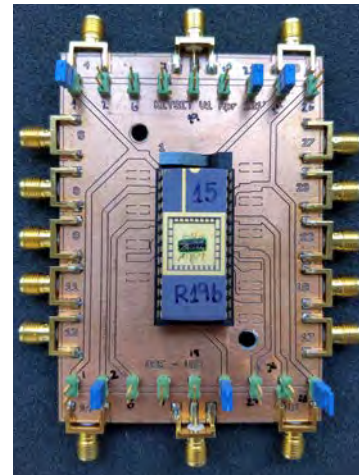
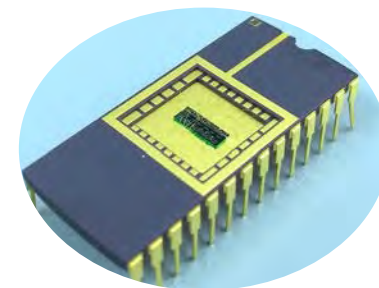
The deposited mass (Δm) can be directly related to the measured frequency change (Δf)

Fabricated Device



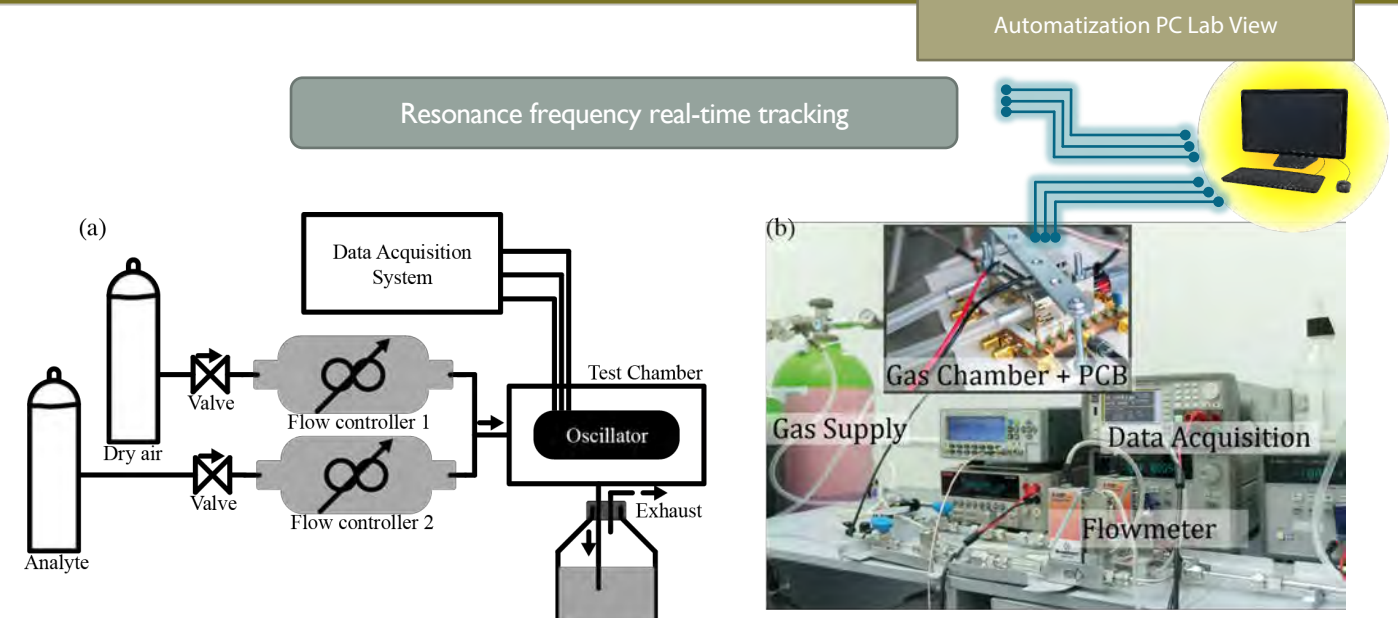
Optical image shows chip-level co-integration: MEMS + circuit

Packaging



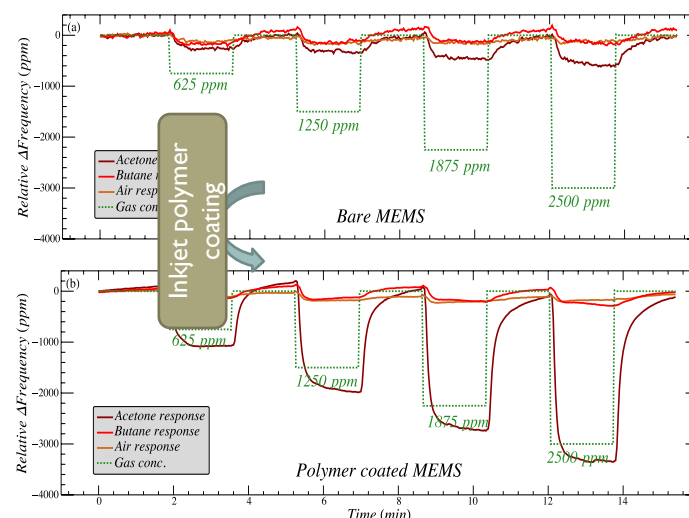
Interconnection to test equipment

VOCs Sensing - Experimental Setup



High-resolution gas mixture setup with two mass flow controllers for accurate analyte injection into test chamber. Resonance frequency is real-time recorded, while monitoring the chamber temperature and acetone concentration sourced.

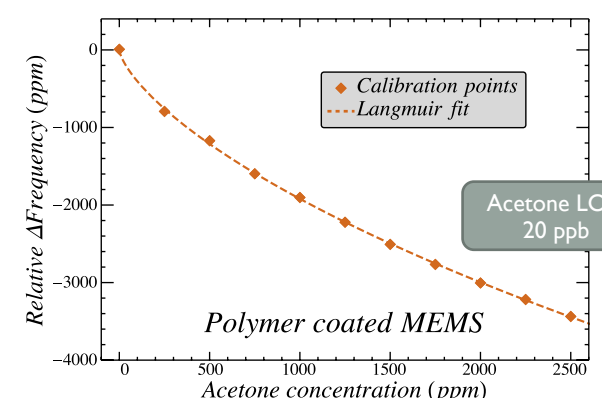
Experimental Results



Recorded resonance frequency time evolution for multiple injections

Acetone response amplified 6X

Butane cross-sensitivity 1:1

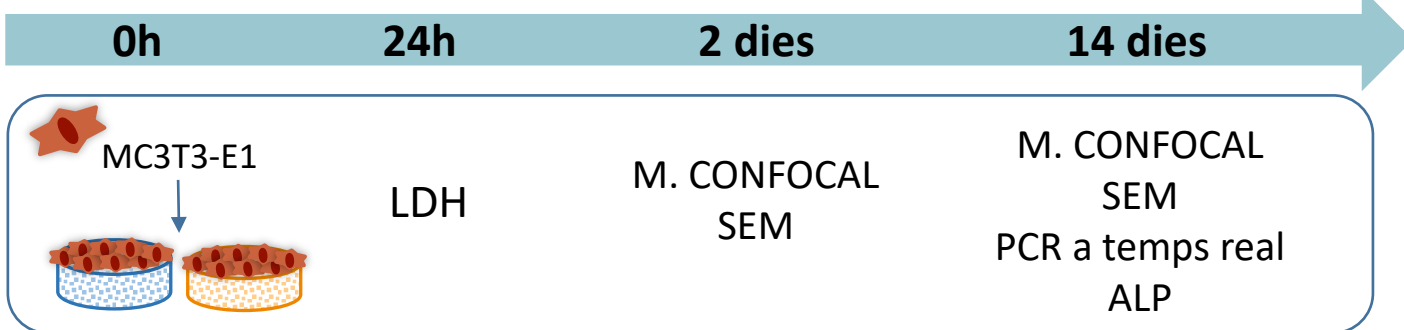
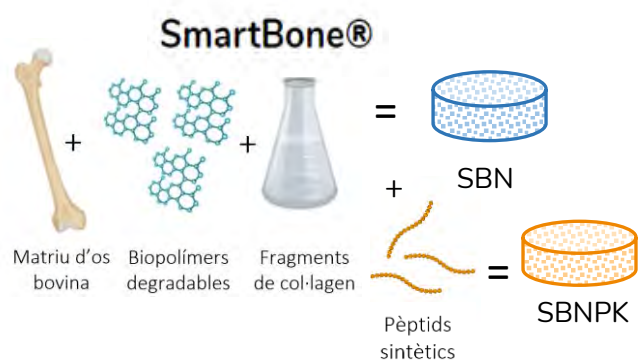


Calibration curve plotting measured frequency shift vs. concentration

Introducció i objectius

- A mesura que la població envellaix cada cop és més difícil tractar amb èxit algunes malalties òssies degeneratives així com defectes del teixit òssi. El tipus de substitut òssi més utilitzat i considerat la millor opció pel fet d'oferir un baix rebuig immunològic és l'autoempelt. No obstant, presenta una sèrie de desavantatges que han donat lloc a l'ús d'altres tipus d'empelts per a la regeneració òssia: al·loempelt, xenoempelt i empelts sintètics.
- L'enfocament actual dins aquest àmbit es centra en crear estructures híbrides combinant components naturals i sintètics. Aquestes estructures compostes tenen l'objectiu d'afavorir una curació ràpida així com la formació d'una interfície entre l'os i l'empelt amb propietats biomecàniques òptimes. Seguint aquestes premisses i els avanços en biomaterials s'ha creat l'empelt SmartBone® (SBN) a partir d'una matriu òssia bovina reforçada amb polímers biodegradables i fragments de col·lagen amb l'objectiu de ser emprat en aplicacions dentals, maxil·lofacials i ortopèdiques.
- Per tant, en aquest treball s'ha duit a terme un estudi sobre la biocompatibilitat i bioactivitat d'aquests empelts òssis, uns control (SBN) i uns modificats amb pèptids sintètics (SBNPK), en un model *in vitro* de preosteoblasts murins MC3T3-E1.

Materials i mètodes



Resultats

1. Citotoxicitat

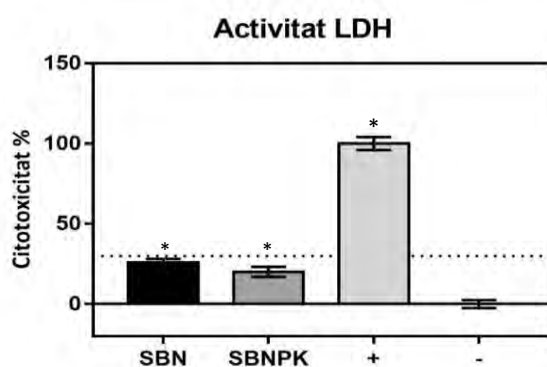


Figura 1. Avaluació de la citotoxicitat de cèl·lules MC3T3-E1 cultivades sobre SBN i SBNPK. El control positiu (+) correspon a la mesura de LDH en el medi de cèl·lules tractades amb 1% de Tritó X-100 i el control negatiu (-) a cultivades sobre plàstic.

2. Proliferació i morfologia cel·lular

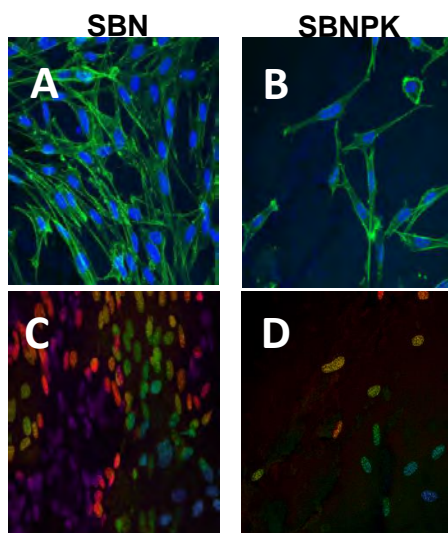


Figura 2. Imatges obtingudes a partir del microscopi confocal després de 14 dies de cultiu en SBN i SBNPK.

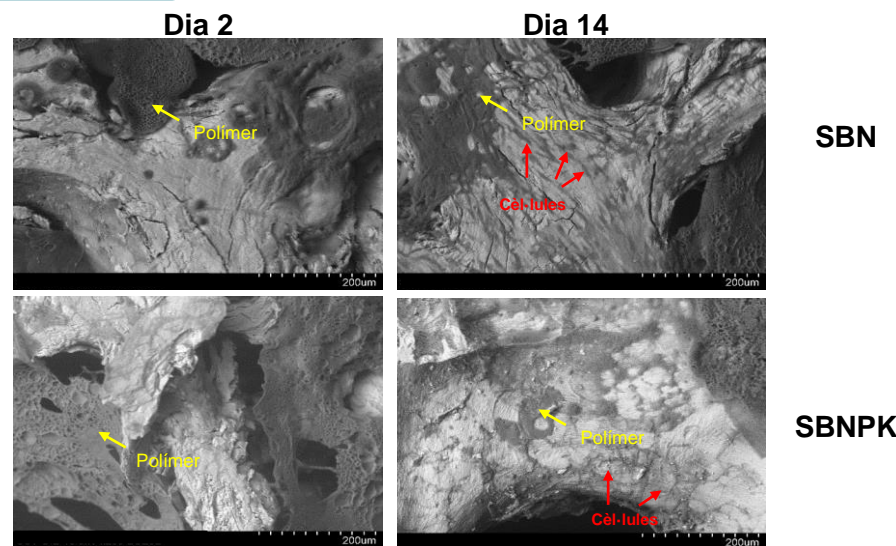


Figura 3. Imatges obtingudes a partir del SEM després de 2 i 14 dies de cultiu.

3. Anàlisi expressió gènica

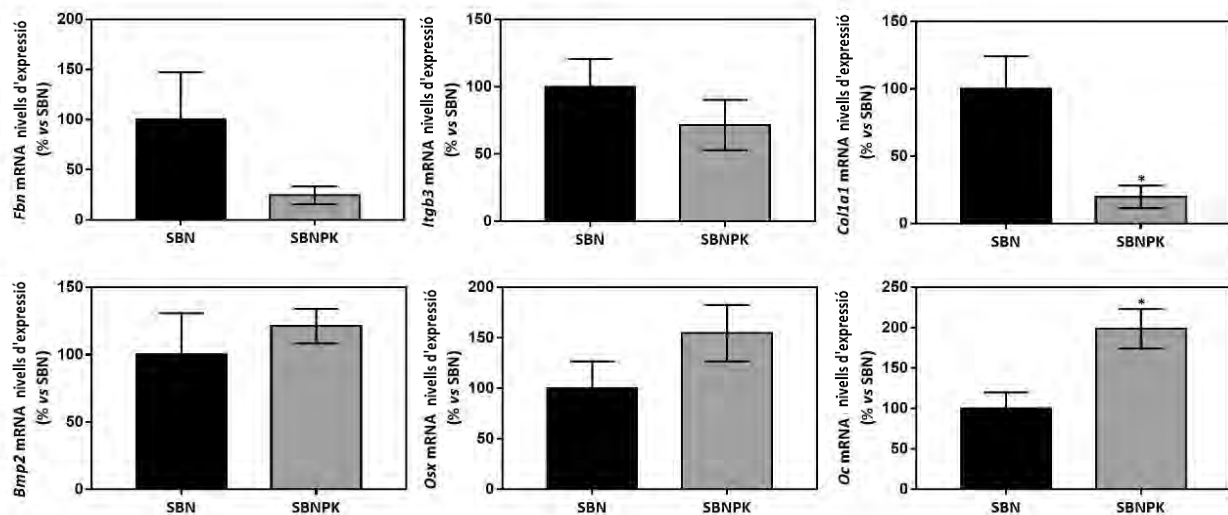


Figura 4. Nivells d'expressió de mRNA de Fbn, Itgb3, Col1a1, Bmp2, Osx i Oc. Els resultats han estat normalitzats amb gens de referència (18S rRNA i GAPDH) i expressats com a percentatge del control (SBN) que representa el 100%.

4. Activitat ALP

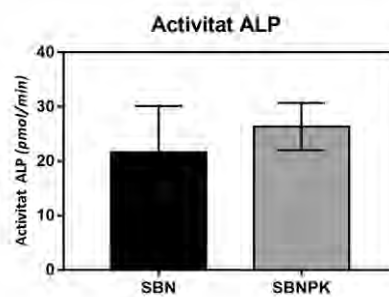


Figura 5. Activitat ALP de les cèl·lules MC3T3-E1 cultivades en SBN i SBNPK durant 14 dies.

5. Concentració RNA total

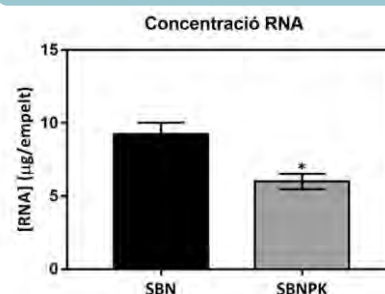


Figura 6. Concentració total de RNA després de 14 dies de cultiu.

Conclusions

- Tant SBN com SBNPK augmentaren l'activitat LDH respecte al control negatiu. Tot i això, no es superà el límit establert com indicador de toxicitat (30%).
- Les imatges del SEM i del microscopi confocal, una major concentració de RNA total i uns nivells més elevats de mRNA de *Col1a1* mostraren una major proliferació en SBN.
- Respecte a la diferenciació osteoblàstica, SBNPK presentà un augment significatiu de mRNA d'*Oc*. L'activitat ALP va augmentar, de forma no significativa, en SBNPK.
- Per tant, el grup SBNPK promou una major diferenciació dels osteoblasts, mentre que el grup SBN presentaria una major adhesió i proliferació cel·lular.

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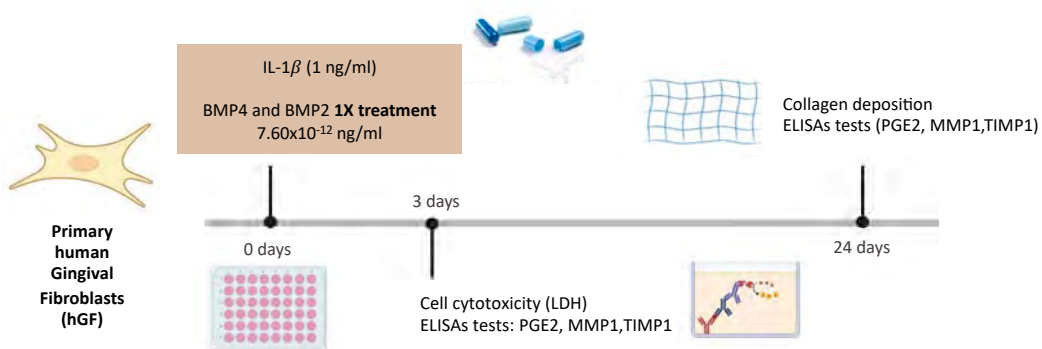
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Background and Aim

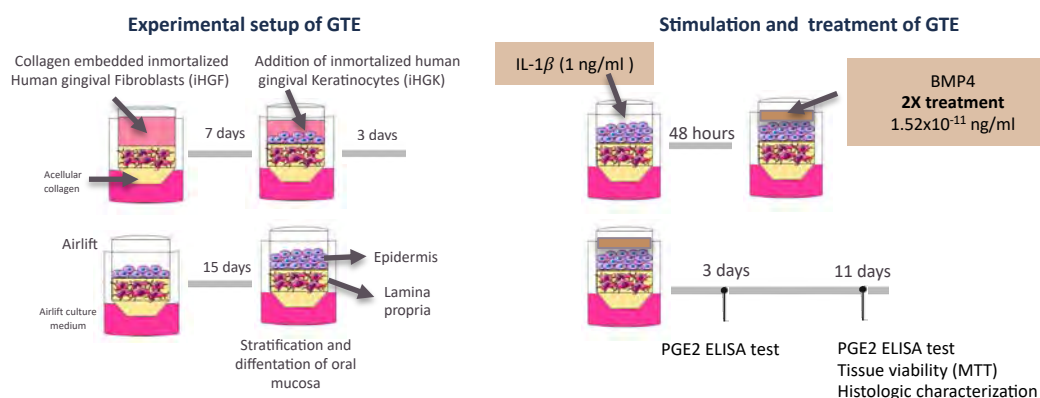
Periodontitis is the most advanced, irreversible and destructive form of periodontal disease, which leads to progressive attachment loss and bone destruction due to this acute (sometimes aggressive) or chronic inflammation. In the present study, we aimed to evaluate the effect of low doses (LD) bone morphogenetic protein-4 (BMP4) and BMP2 micro-immunotherapy (MI) in two in vitro models of periodontal wound healing/regeneration for periodontal applications.

Materials & Methods

hGF cells 2D model



Gingival tissue equivalent (GTE) 3D model



hGF cells 2D model

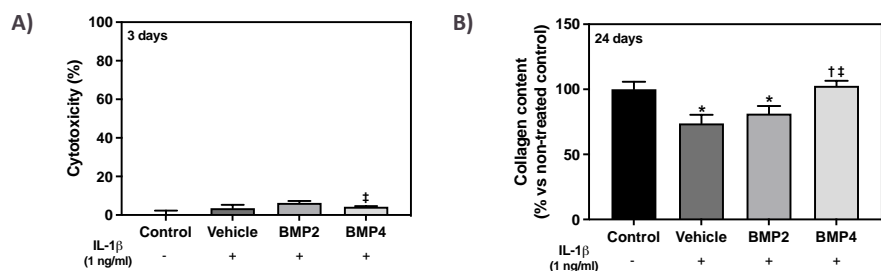


Fig 1. A) Lactate dehydrogenase (LDH) activity, an indicator of cytotoxicity, measured in culture media after 3 days of treatment. Results are presented relative to the LDH activity in the media of cells cultured in tissue culture plastic (negative control was set to 0 % of cell death). B) Collagen deposition in hGF cells after 24 days of treatment. Vehicle (lactose-sucrose) concentration= 7.5×10^6 ng/ml. Data represents the media \pm SEM of six sample replicates (n=6) for each group. $p < 0.05$ and represented with * compared to control, † compared to vehicle, and ‡ compared to LD BMP2.

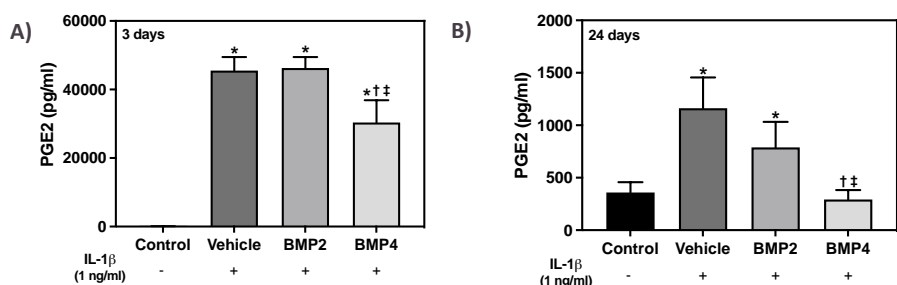


Fig 2. PGE2 release of hGF cells after 3 days (A) and 24 days (B) of treatment. Vehicle (lactose-sucrose) concentration= 7.5×10^6 ng/ml. Data represents the media \pm SEM of six sample replicates (n=6) for each group. $p < 0.05$ and represented with * compared to control, † compared to vehicle, and ‡ compared to LD BMP2.

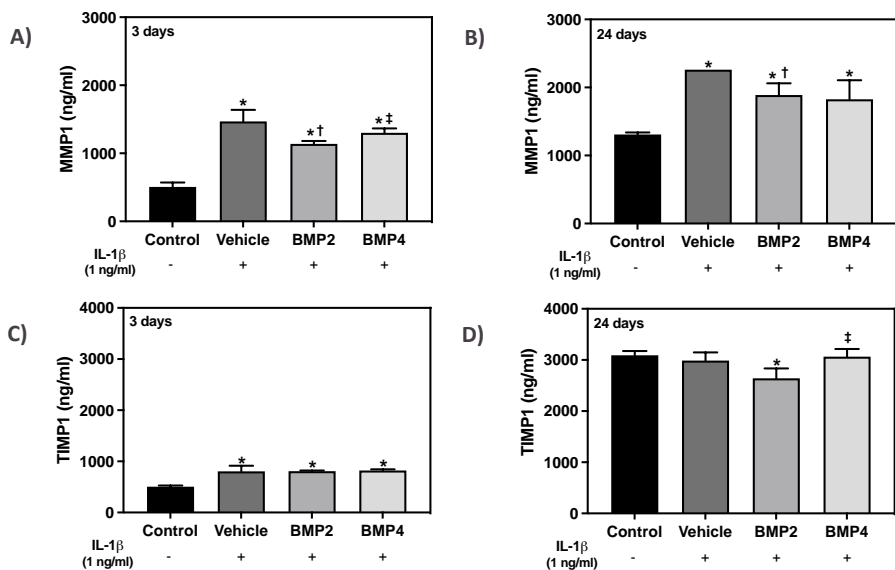


Fig 3. MMP1 protein released to cell culture media after 3 days (A) and 24 days (B) of treatment. TIMP1 protein released to cell culture media after 3 days (C) and 24 days (D) of treatment. Vehicle (lactose-sucrose) concentration= 7.5×10^6 ng/ml. Data represents the media \pm SEM of six sample replicates (n=6) for each group. $p < 0.05$ and represented with * compared to control, † compared to vehicle, and ‡ compared to LD BMP2.

Results

GTE 3D model

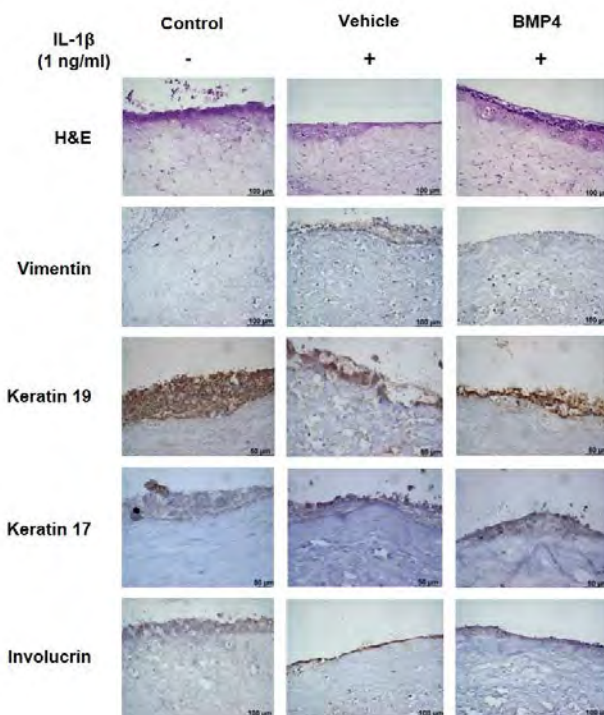


Fig 4: Histologic characterization of GTE. Representative images for each group are shown. H&E staining of GTE 200x; Expression of Vimentin (fibroblasts marker) 200x; Expression of Keratin 19 (epithelial differentiation marker) 400x; Expression of Keratin 17 (epithelial differentiation marker) 400x; Expression of Involucrin (epithelial differentiation marker) 200x. Vehicle (lactose-sucrose) concentration= 15×10^6 ng/ml.

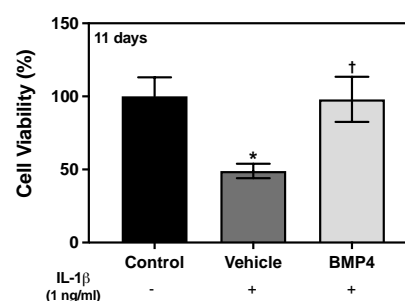


Fig 5. Tissue viability was measured with an MTT test after 11 days of treatment. Positive control was obtained from tissues treated with PBS and was set at 100% of viability. Vehicle (lactose-sucrose) concentration= 15×10^6 ng/ml. Mean \pm SEM (n=2) for each group are represented. $p < 0.05$ and represented with * versus control, and † versus vehicle.

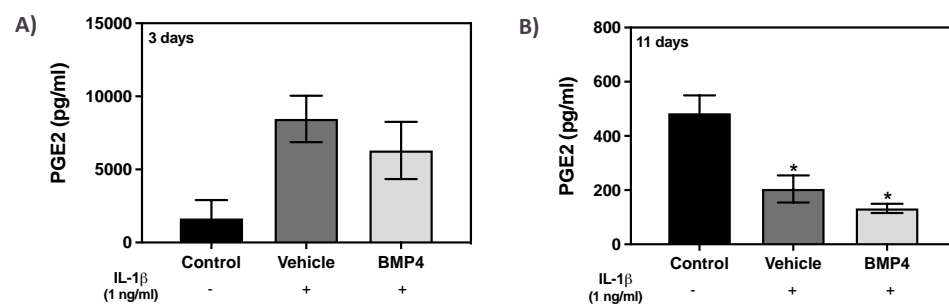


Fig 6. PGE2 release of GTEs media culture after 3 days (A) and 11 days (B) of treatment. Vehicle (lactose-sucrose) concentration= 15×10^6 ng/ml. Data represents the media \pm SEM of six sample replicates (n=6) for each group. $p < 0.05$ and represented with * compared to control, and † compared to vehicle.

Conclusions

1. The present manuscript reports for the first time a beneficial effect of LD BMP4 MI in two in vitro models of periodontitis.
2. LD BMP4 MI treatments presented anti-inflammatory properties and beneficial effects on collagen metabolism on hGF culture.
3. In a more complex 3D model, LD BMP4 recovered tissue viability under inflammatory conditions.
4. We verified the in vitro efficacy of LD BMP4 treatment as a new and promising approach for periodontitis treatment.

Acknowledgements: The project (CONCE 2019 1179) was supported by the Vice Presidency and Ministry of Innovation, Research and Tourism, General Directorate of Innovation and Research, from the Balearic Government (Balearic Islands, Spain), co-funded with ERDF Euroauthor pean Regional Development Fund. Contract to Joana Maria Ramis (MS16/00124) was funded by the Instituto de Salud Carlos III (Madrid, Spain), co-funded with ESF European Social Fund. The authors thank E. Ceresi (University of Balearic Islands, UIB) for the help of histologic characterization.

Uso del MALDI-TOF para el diagnóstico precoz de la ATTRv en muestras de sangre



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Introducción

+ 130 mutaciones gen TTR (chr18q12.1)²

Depósito de fibrillas amiloides derivadas de la acumulación de conformaciones inestables de la proteína transtirretina¹

Amiloidosis hereditaria transtiretina (ATTRv)

Val30Met (Val50Met) foco más frecuente en España, endémica³

Diagnóstico precoz esencial para tratamiento oportuno

Útil para describir biomarcadores en sangre

Permiten desarrollo modelo predictivo (técnicas de aprendizaje automático)

Análisis proteómico

Detección de marcadores indicativos inicio temprano patogénesis y progresión ATTRv y amiloidosis cardíaca wildtype

MALDI-TOF útil para diferenciar muestras e identificar marcadores en biofluidos

Objetivos

- Comparar los niveles de TTR, vitamina A y RBP4 en controles sanos y portadores asintomáticos.
- Validar el uso del MALDI-TOF para el diagnóstico precoz de ATTRv a partir de muestras de suero.
- Crear, evaluar y validar un modelo predictivo basado en el perfil proteómico de los tres grupos (portadores asintomáticos, pacientes y voluntarios sanos).
- Clasificar muestras desconocidas.

Materiales y Métodos

Estudio transversal

- Pacientes
- Portadores asintomáticos
- Sanos (controles voluntarios, prueba genética demostrada)

Foco endémico: Mallorca, España

Variables de estudio

Explicativas

- Fenotipo clínico (momento de la inclusión)
- Tratamiento y estadio de la enfermedad

De respuesta

- Resultados análisis del peptidoma (MALDI-TOF)
- Niveles séricos: TTR, proteína transportadora de retinol (RBP4) y Vitamina A

Otras variables de control

- Datos demográficos (filiación, antecedentes personales y familiares, edad de inicio y de diagnóstico)
- Datos diagnósticos: mutación
- Variables neurológicas: síntomas y complicaciones (examen neurológico o neurofisiológico)
- Variables relacionadas con otros órganos o sistemas (cardíaco, gastrointestinal, renal, oftalmológico, sexual o urinario impotencia)

Muestras

Cuantificación

- TTR y RBP4 (nefelometría; Beckman Coulter)
- Vitamina A (cromatografía líquida de alta resolución)

Preparación MALDI-TOF

- Concentrar y purificar mediante Zip-Tip C18
- Análisis MALDI-TOF

Procesado + análisis datos

- Análisis discriminante lineal (ADL)
- Modelo predictivo (maximizar separación entre grupos)

Resultados

	Sanos	ATTRV30M portadores	ATTRV30M pacientes
n= 89	30	29	30
Edad (media, sd)	41.4 (10.6)	48.0 (15.3)	66.03 (13.0)
Sexo (%mujeres)	60.0%	62.0%	36.0%
Polimorfismo G6S conocido	NA	100%	100%
Herencia materna	NA	25%	70%
Edad de debut (media, sd)	NA	NA	59.8 (13.3)
Edad al diagnóstico (media, sd)	NA	NA	61.7 (14.2)

Reclutamiento de pacientes

Mapa de calor del estudio MALDI-TOF

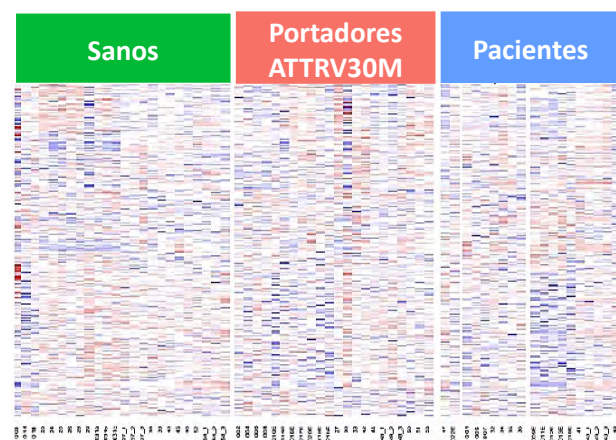


Figura 1: Intensidad de los picos (m/z) obtenidos en los espectros MALDI-TOF de las muestras de suero.

Aplicación de un modelo Discriminante Lineal (DL)

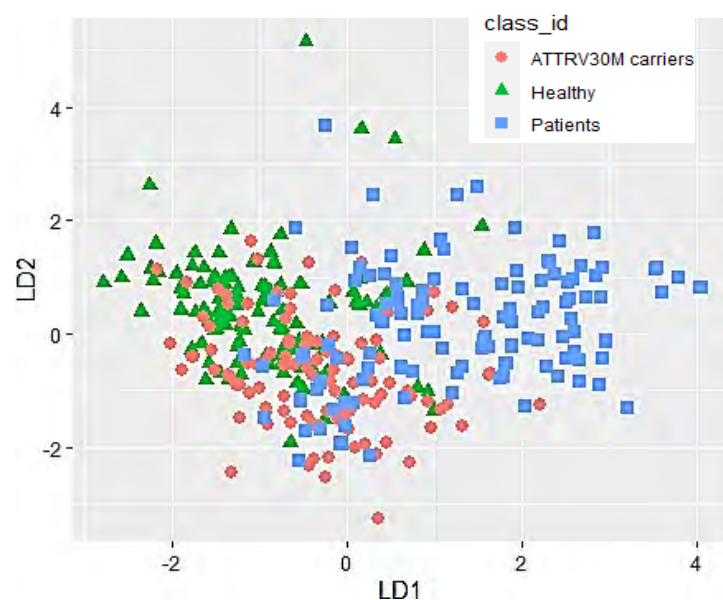


Figura 2: Resultado de la clasificación ADL usando algoritmo de validación cruzada *leave-one-out*. Aparente separación del grupo pacientes respecto a sanos y portadores (precisión del 69.6%). Precisión de clasificación de los tres grupos: 62.9%.

Conclusiones y discusión

Los niveles de TTR son significativamente inferiores ($p < 0.05$) en portadores asintomáticos respecto a controles sanos. No hay diferencias significativas en niveles de vitamina A y RBP4. No se muestran diferencias entre grupos en la representación del estudio MALDI-TOF mediante mapa de calor (Fig.1). El modelo ADL muestra una mayor separación del grupo de pacientes, no concluyente (Fig.2). Se está trabajando en modelos predictivos de aprendizaje automático supervisado no lineales y basados en kernel, para obtener mejor clasificación de las muestras. Se ha empezado un nuevo estudio que consiste en la digestión y purificación de muestras para su posterior análisis MALDI-TOF, con previsión de mejorar la discriminación entre grupos.

Agradecimientos y financiación: El equipo investigador quiere agradecer a todos los individuos que han participado en el estudio. Este proyecto ha sido cofinanciado a través del Programa de Ayudas a la Investigación Independiente de Pfizer (IRR ID#64764667) y Akcea.

1. Ando Y, Coelho T, Berk JL, Cruz MW, Ericzon BG, Ikeda S, et al. Guideline of transthyretin related hereditary amyloidosis for clinicians. *Orphanet J Rare Dis.* 2013; 8: 31– 8.
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Analysis of Spanish university students' healthy habits identifying statistical dependencies in a Bayesian network

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ABSTRACT

Objective: The present study sought to discover the relationships among different features characterizing Spanish university students' habits through a Bayesian network (BN). The set of features with the strongest influence in specific features can be determined. **Methods:** A BN was built from a dataset composed of 13 relevant features, determining the dependencies and conditional independencies from empirical data in a multivariate context. The structure was learned with the *bnlearn* package in R language introducing prior knowledge, and the parameters were obtained with Netica software. Three reasoning patterns were considered to make inferences: *intercausal*, *evidential*, and *causal* reasoning. **Results:** BN determined the different relationships. Through inference several conclusions were achieved, for instance a high probability value of physical activity in low state was obtained when active peers were instantiated to none state, self-rated fitness to fair state, bmi to normal weight, sitting time to moderate, age to 22–25, and gender to woman state. **Conclusions:** Bayesian networks may help to characterize Spanish University students' habits.

KEYWORDS

Alcohol consumption; Bayesian networks; physical activity; screen time

$$P(X_1, \dots, X_n) = \prod_{i=1}^n P(X_i | Pa(X_i))$$

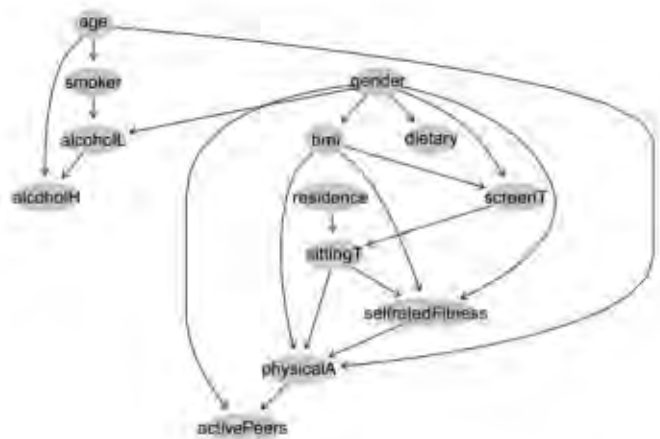


Figure 1. Pattern obtained when hc algorithm (from bnlearn package in R language) performs the search on data with prior knowledge.

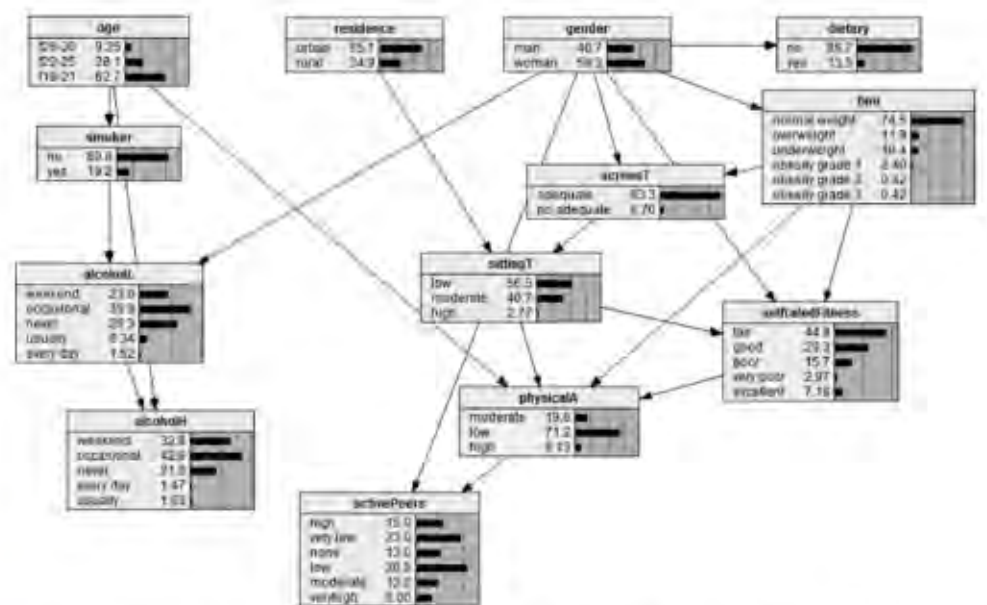


Figure 2. Bayesian network for the study of habits in Balearic university students implemented in Netica software. Probabilities are expressed in percentages. The BN shows an adequate (93.3%) screen time, no (80.8%) smoker, normal weight (74.5%), and low (71.2%) physical activity. It also shows moderate levels of occasional (39.9%) and weekend (23.0%) consumption of low grade alcohol and occasional (42.6%) and weekend (32.0%) consumption of high grade alcohol.

Table 4. Step-by-step instantiations leading to maximization of the likelihood of *physicalA*, where *physicalA* = high in the initial BN without evidence reaches a likelihood of 9.13% expressed in percentage.

Step	Instantiated variable	Value	PhysicalA = high
1	<i>bmi</i>	= obesity grade 2	32.9%
2	<i>activePeers</i>	= moderate	37.4%
3	<i>gender</i>	= woman	44.1%
4	<i>age</i>	= 18–21	44.5%
4'	<i>sittingT</i>	= high	44.5%
4''	<i>selfratedFitness</i>	= good	44.5%

Note. Features such as *age*, *sittingT*, or *selfratedFitness* have the same influence on maximizing physical activity.

Table 5. Step-by-step instantiations leading to maximization of the likelihood of screen time in no adequate state (*screenT* = no adequate) in the initial BN without evidence reaches a likelihood of 6.70% expressed in percentage.

Step	Instantiated variable	Value	Screen = no adequate
1	<i>bmi</i>	= obesity grade 3	50.00%
2	<i>sittingT</i>	= high	85.10%
3	<i>gender</i>	= woman	91.90%
4	<i>residence</i>	= urban	92.2%

Table 7. Step-by-step instantiations leading to maximization of the likelihood of *bmi* in overweight state (*bmi* = overweight) in the initial BN without evidence reaches a likelihood of 93.3% expressed in percentage.

Step	Instantiated variable	Value	bmi = overweight
1	<i>selfratedFitness</i>	= very poor	28.8%
2	<i>gender</i>	= man	39.0%
3	<i>sittingT</i>	= moderate	41.3%
4	<i>screenT</i>	= no adequate	42.0%



IdISBa

ACEPTACIÓN DEL SEGUIMIENTO MEDIANTE LA APLICACIÓN REDSCAR© EN PACIENTES REALES

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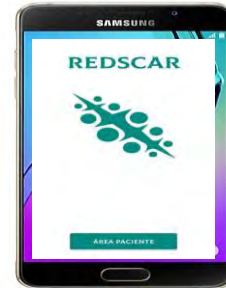
Este trabajo parte del proyecto de I+D+i PID2020-113870GB-I00, financiado por MCIN/AEI/10.13039/501100011033/



TELEMEDICINA



SATISFACCIÓN MÉDICO-PACIENTE



REDSGAR© INFECCIÓN HERIDA QUIRÚRGICA



OBJETIVO: El objetivo del estudio es valorar el nivel de satisfacción de los sujetos sometidos a seguimiento telemático a través de la aplicación RedScar© mediante un cuestionario validado sobre satisfacción en telemedicina.

RESULTADOS: Participación encuesta satisfacción: 37 pacientes (82,2%)

Preguntas	N = 37
Dificultad para cargar la imagen y analizarla	43,2%
Tiempo uso medio	3,7 min
Menor tiempo al realizar el seguimiento telemático que acudiendo a la consulta	94,3%
Reducción costes	100%
Detección de infección más rápida mediante la app	82,9%
Fiabilidad del seguimiento telemático	80,6%
Preferencia por el seguimiento telemático	54,3%
Volverían a utilizar una app para el seguimiento de su estado de salud	85,3%

CONCLUSIONES: A la mayoría de los pacientes les parece fiable el seguimiento telemático aunque sólo un 54,3% preferirían este seguimiento al presencial, siendo el principal motivo los errores en el funcionamiento de la app. Corregir estos errores, mejorando el rendimiento de la aplicación aumentaría el grado de aceptación entre los pacientes.