To attain a successful ECM analogue scaffold, there are several design and material criteria that must be satisfied involving the mimicking of topographical features and geometry on the macro-, micro- and even at nanoscale levels, because each influences cell response to the scaffold.

In the last years, a successful approach has been represented by the use of composite scaffolds obtained by a combination of phase inversion, salt leaching, filament winding technology. These techniques enable obtaining porous scaffold with controlled micro and macro porosity able to influence positively mechanical properties and cell interactions. In particular, composite materials based on biodegradable polymers (i.e. poly-ε-caprolactone) endowed with intrinsically bioactive particles (i.e. calcium phosphates) and/or macromolecules (i.e Hyaluronic Acid), offers the possibility to realize a strong bond with natural tissues through more bioactive, structurally and mechanically efficient interfaces, firstly enhancing the capability of the substrate to form new extracellular matrix (ECM) and assuring a more rapid and efficacious integration to the implant site. However, some limitations of traditional process impose to identify innovative strategies for fabricating micro and nanostructures structures.

In this context, interesting approaches based on the assembly of basic components or building blocks endowed with molecular signals are powerfully emerging to form hierarchically complex structures, able to accurately recapitulate the functional properties of natural complex structures. For connective tissue regeneration (bone, ligaments, meniscus) composite scaffolds are obtained by phase inversion, salt leaching and RP technique to modulate mechanical properties and cell interactions.

Design of bioactive scaffolds for bone regeneration with appropriate porosity and high pores interconnectivity could be obtained by using Poly(ε-caprolactone) reinforced with Calcium Phosphates particles and PLA fibres.

Ester of Hyaluronic Acid reinforced with degradable fibres were processed by composite technology, phase inversion and salt leaching technique to obtain scaffolds for meniscus regeneration. In vivo results demonstrated the possibility to regenerate the meniscus by using an appropriate scaffolds. Imaging and rapid prototyping technologies are implemented to design a “custom made” meniscus scaffold. A critical discussion on the advantages of new approaches has been performed by proposing strategies based on composite to the assembly of elementary components such as fibres implemented through modified electrospinning or sintering techniques.

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tion. Dye-loaded NSPs were isolated by ultracentrifugation and filtration through 0.22μm filters and their morphology and size distribution was characterized by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS), respectively. Formulations of NR-NSPs dispersed in PBS and in 1% w/v HydroxyPropyl Methyl Cellulose (HPMC) gel made in PG:water 80:20 v/v, with and without 0.2 M Azone were evaluated for NSP morphology (TEM) and release profiles (diffusion through dialysis membranes). The in vitro skin penetration of NR via the above formulations (n=6) was evaluated in human cadaver skin with the aid of static vertical Franz Diffusion cells. In vivo skin penetration studies (n=6) were conducted in a domestic pig with the aid of HillTop Chambers that served to enclose the formulations and confine them to different sites on the back of the pig. Controls included NSPs only and solution of NR in PG:water with and without 0.2 M Azone. The extent and amount of skin penetration of NR in human and porcine skin was evaluated by cryosectioning the treated skin areas and determining the NR penetration into the vertical skin sections by fluorescence microscopy and dye intensity quantification. Analysis of variance was used for statistical analysis and treatments were considered significant if p<0.05.

Results and Discussion

The NSPs demonstrated a spherical morphology (FIG.2) with a relatively narrow size distribution centered around 55 nm (FIG.3). Dispersion of the NSPs in the HPMC gel produced a uniform and stable formulation with insignificant changes in the size and morphology of the NSPs (FIG.4). In addition, the HPMC gel served as a suitable formulation for the co-existence of NSPs and the chemical penetration enhancer Azone. The binding efficiency of NSP to NR was found to be ~ 65% w/w. In vitro release of NR from the NSPs occurred with an initial burst release (~ 5%) followed by a steady fickian diffusion of the NR (~45% release in 24h) (FIG.5). Dispersion of the NR-NSP in HPMC resulted in for-
mulations with high viscosity, providing close contact of the NSPNR with skin during and after application. In vitro skin penetration (FIG.6 A-D) of NR via the NSP in the epidermis, upper and lower dermis was 5, 7 and 3.5-fold higher than the corresponding NR solution in PG:Water (80:20 v/v) (p<0.05). NR penetration into the upper and lower dermis via the NR-NSP-HPMC gel was 1.4- and 1.8-times higher (p<0.05) than the aqueous formulation of NRNSP. Azone further increased the dermal penetration of NR-NSP-HPMC by 2-fold. Similar results were obtained with the in vivo studies (FIG.6-E, images not shown here), with 2.3-fold (p<0.05) increase in NR permeation into the stratum corneum/epidermis layers when delivered via NSP compared to PG:water. The combined NR-NSP-HPMC gel formulation was found to deposit 1.4- (p<0.05) higher amount of NR in the stratum corneum/epidermis than the aqueous NR-NSP aqueous dispersion. The combination of Azone with the NR-NSP-HPMC formulation demonstrated the highest skin penetration of NR, with a 2.5 fold deposition as compared to NR-NSP-HPMC gel and a 4.5-fold (p<0.05) higher deposition than the formulation of NR in PG:water alone.

References


Conclusion

Tyrosine-derived nanospheres formulated as aqueous or gel systems significantly enhanced the in vitro and in vivo skin penetration of NR, compared to the corresponding non-particulate formulation. The in vitro studies demonstrated the enhanced delivery potential of these nanocarriers to the epidermal-dermal junction and deeper skin layers, where psoriasides and other diseases originate (FIGs.6, A-D). Formulation of the NSPs in HPMC gel significantly increased their efficacy due to higher skin penetration and a viscosity that provided close skin contact and superior hydration. While the combination of Azone with the NSP significantly enhanced the skin deposition of NR (FIG.6, D and E), the cytotoxicity of Azone limits its use in higher concentrations. Thus, the gel formulation of the NSP, alone or in combination with lower concentrations of an enhancer can be used for delivery of lipophilic agents to deeper skin layers.