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**Title:** High performance functional bio-based polymers for skin-contact products in biomedical, cosmetic and sanitary industry.

**Acronym:** PolyBioSkin

**Grant Agreement No:** 745839

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Abstract

PolyBioSkin aims to produce innovative cosmetic, biomedical, and absorbent hygiene applications that are bio-based (90% or more) and biodegradable. Specifically, the PolyBioSkin target demonstrators are baby diapers and femcare sanitary pads, facial beauty sheet masks, and an advanced wound dressing application.

This report provides a detailed overview over the key materials that will be considered and tested for application in the project, which will be involved in the design of the final product prototypes.
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<tr>
<td>AA</td>
<td>Adipic acid</td>
</tr>
<tr>
<td>AGU</td>
<td>Anhydroglucose unit</td>
</tr>
<tr>
<td>AHP</td>
<td>Absobent hygiene product</td>
</tr>
<tr>
<td>BDO</td>
<td>1,4-butandiol</td>
</tr>
<tr>
<td>Bio-PBS</td>
<td>Bio-based poly(butylene succinate)</td>
</tr>
<tr>
<td>Bio-PET</td>
<td>Bio-based polyethylene terephthalate</td>
</tr>
<tr>
<td>CMC</td>
<td>Carboxymethyl cellulose</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>Carbon to nitrogen ratio</td>
</tr>
<tr>
<td>CNs</td>
<td>Chitin nanofibrils</td>
</tr>
<tr>
<td>CoA</td>
<td>Coenzyme A</td>
</tr>
<tr>
<td>dcw</td>
<td>Dry cell weight</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's Modified Eagle Medium</td>
</tr>
<tr>
<td>DNA</td>
<td>Desoxyribonucleic acid</td>
</tr>
<tr>
<td>DS</td>
<td>Degree of substitution</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier-transform infrared spectroscopy</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally recognised as safe</td>
</tr>
<tr>
<td>LA</td>
<td>Lactic acid</td>
</tr>
<tr>
<td>MCA</td>
<td>Monochloroacetic acid</td>
</tr>
<tr>
<td>mcl-PHAs</td>
<td>Medium chain length polyhydroxyalkanoates</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>OTR</td>
<td>Oxygen transfer rate</td>
</tr>
<tr>
<td>P(3HB)</td>
<td>Poly(3-hydroxybutyrate)</td>
</tr>
<tr>
<td>P(3HD-co-3HDD)</td>
<td>Poly(3-hydroxydecanoate-co-dodecanoate)</td>
</tr>
<tr>
<td>P(3HHp)</td>
<td>Poly-3-hydroxyheptanoate</td>
</tr>
<tr>
<td>P(3HO)</td>
<td>Poly(3-hydroxyoctanoate)</td>
</tr>
<tr>
<td>P(3HX)</td>
<td>Poly(3-hydroxyhexanoate)</td>
</tr>
<tr>
<td>PBAT</td>
<td>Poly(butylene adipate-co-terephthalate)</td>
</tr>
<tr>
<td>PBS</td>
<td>Poly(butylene succinate)</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>PBSA</td>
<td>Poly(butylene succinate) adipic acid copolymer</td>
</tr>
<tr>
<td>PBSL</td>
<td>Poly(butylene succinate) lactic acid copolymer</td>
</tr>
<tr>
<td>PE</td>
<td>Polyethylene</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PET</td>
<td>Polyethylene terephthalate</td>
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<tr>
<td>PHAs</td>
<td>Polyhydroxyalkanoates</td>
</tr>
<tr>
<td>PLA</td>
<td>Poly(lactic acid)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PP</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>SA</td>
<td>Succinic acid</td>
</tr>
<tr>
<td>SAP</td>
<td>Superabsorbent polymer</td>
</tr>
<tr>
<td>scl-PHAs</td>
<td>Short chain length polyhydroxyalkanoates</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>Tm</td>
<td>Melting temperature / melting point</td>
</tr>
<tr>
<td>Tg</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>TA</td>
<td>Terephthalic acid</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TPS</td>
<td>Thermoplastic starch</td>
</tr>
<tr>
<td>WP</td>
<td>Work package</td>
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1 Introduction

The PolyBioSkin project aspires to engineer (a) a bio-based and biodegradable diaper and a sanitary femcare pad, consisting of an anti-microbial bio-based and biodegradable topsheet beneficial to the skin and a bio-based and biodegradable superabsorbent layer, (b) novel facial beauty sheet masks based on films made form bio-based and biodegradable polymers and impregnated with biomolecules beneficial for the skin, and (c) bio-based and biodegradable nano-structured non-woven textiles for wound dressings. Specifically, the consortium aspires to achieve a level of 90% bio-based content (established on the basis of accepted standards such as ASTM D6866 or the new EN 16785-1) for all applications, and the biodegradability target will be in conformity with the harmonised standard EN 13432/14995, i.e. industrial compostability. The selection of bio-based materials for the project combines formulations based on engineered biopolymers, like poly(lactic acid) (PLA), with naturally available ones, like polyhydroxyalkanoates (PHAs) or chitin. There are already efforts to increase the use of polymers such as PLA in substitution of conventional fossil-based and non-biodegradable commodity polyolefins such as polyethylene (PE) in applications where improved levels of biocompatibility are useful. PolyBioSkin will drive this development by adding chitin nano-fibrils to PLA in order to provide PLA films with excellent anti-microbial properties to avoid skin irritations.

The highly versatile group of emerging biopolymesters commonly subsumed under the name of polyhydroxyalkanoates (PHAs) can be synthesised directly in the cells of a number of microorganisms and the exact polymer structure and molecular weight can vary greatly depending on the microorganism nature and culture conditions. As such, PHAs structure can be different in terms of content of comonomers (3-hydroxybutyric acid, 4-hydroxybutyric acid, 3-hydroxyvaleric acid, etc.) or molecular weight, which in turn can lead to flexible or rigid plastics and to different possibilities of processing in conventional industrial machines. Among the commercially available PHAs, most are produced by Gram-negative bacteria. Despite their unique biocompatibility and even, in some cases, inherent antibacterial properties, PHAs from Gram-positive bacteria are still not commercially utilized. Especially in the case of wound dressings, such new materials could help to avoid immune reactivity and maximise skin regeneration potential.

Chitin is a polysaccharide present in the skeletons of insects and the shells of crustaceans and readily available from food industry processing waste (for instance sea food waste). Chitin and its derived biopolymer chitosan have shown excellent techno-functional properties in different fields, for example for edible coatings with good gas barrier properties, antimicrobial properties for wound care, skin hydration and repairing in cosmetic application or biostimulants for plants. In its nanofibrilic form, chitin has been reported to be a potent skin inflammation suppressant to be applied, for example, against atopic dermatitis. This feature is of huge relevance for all skin-contact applications pursued in the project.

Furthermore, natural superabsorbent cores based on modified cellulose and starch will be designed to substitute the conventionally used petrochemical acrylic absorbents used widely today in absorbent hygiene products such as diaper and femcare pads.

By replacing conventional fossil-based and non-biodegradable materials with a range of suitably engineered biomaterials that are bio-based and biodegradable at least under conditions of industrial composting, PolyBioSkin aspires to pioneer the transition of high-performance applications in the
multi-billion biomedical, cosmetics and absorbent hygiene products to bio-based alternatives which feature improved performances due to leveraging the unique attributes of the selected biopolymers in terms biocompatibility and other properties such as anti-inflammatory, anti-septic, and anti-oxidant effects. The project also aims to open the door for a more sustainable end-of-life treatment for these very high-volume product categories by harnessing the selected biopolymers’ biodegradability features, suggesting promising alternatives to the currently practised landfilling and incineration.

This report will provide a detailed introduction to the polymers preselected for use in the PolyBioSkin target applications. It provides the basis for the work undertaken in subsequent work packages, where, based on results and the insights won throughout the project, the final formulations will be determined. It will also provide basic insights into the commercial competitiveness of the envisaged bio-based and biodegradable target applications. Further details on the properties of the materials selected for each specific application will be included in future deliverables associated to WP1 Specifications of PolyBioSkin target applications and selection of bio-raw materials for skin, WP2 Bioplastic formulations for films and fibers, WP3 Bio-based polymer fibers and nonwovens and WP4 Bio-based micro- and nano-structured nonwovens, currently under preparation.

2 Specification of biopolymers selected for PolyBioSkin applications

This chapter provides a comprehensive overview over the pre-selection of materials and substances that will be considered within the PolyBioSkin project. The selection focussed on specific inherent properties rendering them suitable for the purpose of engineering the PolyBioSkin skin-contact AHP, cosmetic, and wound dressing applications, and it is based on the project’s industrial and R&D partners’ extensive experience with the testing and processing of these materials and substances.

2.1 Technical bipolymers

2.1.1 Polyhydroxyalkanoates (PHAs)

PHAs are polyesters composed of several units of hydroxyalkanoate monomers linked to each other through ester linkages. Figure 1 shows the general structure of PHAs, where ‘n’ is the number of monomer units in each polymer chain, which varies between 100 and 30000; ‘R’ is the side chain that includes alkyl groups with 1 to 13 carbons and ‘x’ in the main chain ranges from 1 to 4.

![Figure 1 General formula for PHAs, R: Alkyl group C1-C13, x: 1-4, n: 100-30000.](image-url)
Numerous microorganisms synthesize PHAs by the fermentation of a carbon source and then accumulate them as intracellular carbon reserve inclusion bodies. In order to accumulate PHAs, in most bacteria, an excess supply of carbon and limitation of nitrogen, phosphorus, oxygen or magnesium is required. These limiting conditions result in a decrease in cell growth and division, and a redirection of their metabolism towards the biosynthesis of the PHAs (Jurasek et al., 2004). The stored PHAs can be degraded by intracellular depolymerases and metabolized as a carbon and energy source when needed (Byrom et al., 1994).

The properties of PHAs vary depending on the distance between ester groups in the molecule, the structure of the side groups and the number of monomer units in the polymer chain. For example, the length of the side chain and its functional group has a direct effect on the polymer’s physical properties such as flexibility, crystallinity, melting point and glass transition temperature (Volvoa et al., 2004). The nature and proportion of the PHA monomers are influenced by the type and relative quantity of carbon sources supplied to the growth media, the organism used and the culture conditions provided (Ojumu et al., 2004). For instance, based on growth conditions and the used microorganism, the molecular weight of the polymers can vary from $2 \times 10^5$ to $3 \times 10^6$ daltons (Byrom et al., 1994).

PHAs can be either thermoplastic or elastomeric materials with variable mechanical, thermal stability and durability properties. They are water insoluble and impermeable to oxygen (Chen et al., 2010). Due to the stereospecificity of the PHA synthase, all the hydroxyalkanoate monomers incorporated in the polymer are in the R(−) configuration, resulting in an optically pure polymer (Zinn et al., 2005). Additionally, they are biodegradable; hence, they can be degraded and metabolized by microbes and by enzymes within the human body; biocompatible, they do not generate toxic by-products and some PHAs are piezoelectric, a property known to stimulate cell growth (Philip et al., 2007).

Based on the number of carbon atoms in the monomer units PHAs can be divided in two main different types: the short chain length polyhydroxyalkanoates (scl-PHAs), which consist of C3-C5 atoms, and the medium chain length polyhydroxyalkanoates (mcl-PHAs) consisting of C6-C14 atoms (Ojumu et al., 2004). Also, it has been observed that some organisms produce copolymers including both scl and mcl monomers, these are referred to as scl-mcl PHAs. These types of PHAs are a consequence of the PHA synthase substrate specificity, which accepts precursors of a certain range of carbon length (Rehm et al., 2001).

Poly(3-hydroxybutyrate), P(3HB), the simplest and most common example of the scl-PHAs, is a highly crystalline, brittle, stiff and piezoelectric material with a melting temperature of 1770°C, glass transition temperature of 40°C, tensile strength of 40 MPa and elongation at break of 1-6%. Its biological properties include, complete biodegradability, water resistance, high biocompatibility and a suitable substrate for tissue engineering which enhances cell adhesion, migration, proliferation and differentiation functions (Saad et al., 1999). The mcl-PHAs such as poly(3-hydroxyhexanoate), P(3HX) or poly(3-hydroxyoctanoate), P(3HO), are thermoplastic elastomers with melting points, $T_m$, ranging between 40-60°C and glass transition temperatures, $T_g$, ranging between -50 to -25°C. The mcl-PHAs have lower crystallinity with higher flexibility and softness. They are more thermally stable than scl-PHAs with an elastomeric nature which increases with the length of the side chain. These are also biodegradable, water resistant and biocompatible, which could be utilized in medical implants, such as scaffolding for the regeneration of arteries and nerve axons (Witholt et al., 1999).
Table 1 compares the physical properties of P(3HB), a scl-PHA, P(3HO), a mcl-PHA, and polypropylene, a commonly used synthetic polymer. The crystallinity and tensile strength of P(3HB) are similar to those of propylene, however, the elongation to break is significantly lower than that of propylene. On the other hand, the Young’s modulus and elongation to break values for P(3HO) are comparable to those of propylene and dissimilar in terms of crystallinity and tensile strength. The scl-mcl PHA copolymers have properties intermediate between scl and mcl PHAs (Nomura et al., 2004).

<table>
<thead>
<tr>
<th>Properties</th>
<th>scl–PHAs (P(3HB))</th>
<th>mcl-PHAs (P(3HO))</th>
<th>Polypropylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>175</td>
<td>49</td>
<td>176</td>
</tr>
<tr>
<td>Glass-transition temperature (°C)</td>
<td>15</td>
<td>-36</td>
<td>-10</td>
</tr>
<tr>
<td>Crystallinity (%)</td>
<td>81</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Young’s modulus (GPa)</td>
<td>3.5</td>
<td>1.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Tensile strength (MPa)</td>
<td>40</td>
<td>9</td>
<td>34.5</td>
</tr>
<tr>
<td>Elongation at Break (%)</td>
<td>6</td>
<td>276</td>
<td>400</td>
</tr>
</tbody>
</table>

Table 1 Comparison of the physical properties of P(3HB), a scl-PHA, P(3HO), a mcl-PHA and polypropylene (Ojumu et al., 2004, Rai et al., 2010).

Nowadays, most of the studies focus on the development of different types of copolymers for the production of tailor made materials to suit different applications. For example, one of the most commonly produced copolymers that is commercially available, is P(3HB-co-3HV). This copolymer is more ductile, elastic and flexible than P(3HB) due to the presence of the hydroxyvalerate groups (El-Hadi et al., 2002). Moreover, it has been reported that the increment in hydroxyvalerate units results in the lowering of the melting temperature, crystallinity and tensile strength of the polymer, but an increment in the flexibility, impact strength and ductility of the material (Conti et al., 1996). These approaches make PHAs suitable for a wide range of applications and a promising class of new emerging biomaterials (Akaraonye et al., 2010).

Biodegradability of PHAs

One of the most valuable properties of PHAs is their biodegradability in natural environments. PHAs are degraded completely to carbon dioxide and water under aerobic conditions and with methane formation under anaerobic conditions (Volvoa et al., 2006). PHAs can be degraded by depolymerases present in microorganisms and enzymes present in the blood and animal tissues (Jendrossek et al., 2002). It has been demonstrated that the main factors that influence the PHA biodegradation are the stereoconfiguration of the monomers, the crystallinity, the molecular mass and the chemical composition of the polymer. Only ester linkages of monomers in the (R)-configuration are hydrolysed by the depolymerases (Volvoa et al., 2004). In terms of the molecular mass, high molecular mass polymers are degraded more slowly than low molecular mass polymers. Quinteros et al, studied the relation between the alkyl side chain length and biodegradability, showing an increment in the
degradation rates with the side chain length (Quinteros et al., 1999). Additionally, Abe et al. observed that an increment in the polymer crystallinity resulted in a reduction of the degradation rate and that the depolymerases first hydrolyse polymer chains in the amorphous phase, followed by crystalline phases, with a depolymerisation rate 20 times higher for the amorphous phase than the crystalline phase (Abe et al., 1999). Volvoa et al. studied PHA biodegradation and observed that the first week involved the degradation of the amorphous phase resulting in the disruption of the polymer chain. This resulted in the formation of tetramers, dimers and monomers, with a decrease in the molecular mass. Finally, the polymer lost its mass and this process could take from months up to 2-3 years, depending to the polymer properties and environmental conditions (Volvoa et al., 2004). Weng et al. studied the influence of chemical structure on the biodegradability of P(3HB)-co-(3HV). Results showed that an increment in the hydroxyvalerate subunits resulted in an increment on the biodegradation and that the biodegradation occurred by enzyme catalysed erosion from the surface to the interior (Weng et al., 2011). Rai et al studied the degradation behaviour of the P(3HO) homopolymer films. In contrast to other amorphous polymers such as PLGA which showed bulk degradation, P(3HO) films showed only around 15% of degradation in DMEM media after 3 months and this is possibly due to the hydrophobic nature of the polymer and it’s semicrystalline structure (Rai et al., 2011).

**Biocompatibility of PHAs**

One of the fundamental requirements for a material to be suitable for medical applications is that it should display adequate biocompatibility. In response to the material composition and degradation products, the host body can release either pro- or anti-inflammatory mediators, which can eventually cause initial acute inflammation, followed by chronic inflammation and possible ultimate rejection of the biomaterial. In addition to the chemical and molecular structure, different parameters are known to have an influence on the biocompatibility of the material such as the size, porosity, shape and surface topography (Zhao et al., 2003). Among the different parameters, the haemocompatibility of the material is one of the main aspects that will determine the suitability of the material for medical applications. The material should not cause thrombosis, embolisms, antigenic response and destruction of plasma proteins. The haemocompatibility of a material can be evaluated by the haemostasis system, which can be studied by different parameters such as the morphology of the attached platelets and complement activation. Sevastianov et al studied the haemocompatibility of P(3HB) and P(3HB-co-3HV) films produced from R. eutropha through the determination of relative number and morphology of adherent platelets, complement activation and coagulation system activation. PHA films in contact with blood did not activate the haemostasis system at the level of cell response, but they did activate the coagulation system and the complement reaction. Further experiments, which included purification of the produced polymers showed that resulting P(3HB) and P(3HB-co-3HV) were suitable to be used in contact with blood and that the presence of lipopolysaccharides from the producer bacteria was the factor activating the haemostasis systems (Sevastianov et al., 2001, Sevastianov et al., 2003). One of the main reasons of PHA biocompatibility is the presence of PHA monomer units in human plasma and tissues. It has been demonstrated that the monomer found in the P(3HB) polymer, (R)-3-hydroxybutanoic acid, is present at concentrations of 3-10 mg in 100 ml of blood in healthy humans. Additionally, the presence of low molecular weight forms of P(3HB) have also been detected in lipoprotein fractions of human tissues (Hocking et al.,
1994, Nelson et al., 1981). However, although a material can be biocompatible, presence of impurities derived from the method of production and extraction can affect the final biocompatibility of the material, as previously described (Williams et al., 1995).

**Biosynthesis of PHAs**

PHA biosynthesis has been intensively studied over the years. Different metabolic pathways were described and the particular pathway used for PHA production was found to depend on the particular metabolic pathways that are operating in a particular microorganism and the carbon source provided. PHA biosynthesis can be divided in two main steps. The first step involves the synthesis of hydroxyacyl-CoA, the PHA linked monomer units. The second step involves a reaction catalysed by the PHA synthase which use the hydroxyacyl-CoA units as substrates and catalyses their polymerization into PHAs with the concomitant release of CoA (Rehm et al., 2001). The synthesis of the 3-hydroxyacyl units can occur mainly by three different pathways (Figure 2) (Kim et al., 2007, Doi et al., 1990, Poirier et al., 1995, Steinbüchel et al., 1991). Two of the pathways involve the production of PHAs using carbohydrates as a carbon source and a third using fatty acids.

**Pathway I**

- **Carbon source** (Sugars)
- TCA cycle
- Acetyl-CoA
- PhaA
- Acetoacetyl-CoA
- PhaB
- (R)-3-Hydroxybutyryl-CoA
- PhaC

**Pathway II**

- **Fatty acid degradation** (K-Carotenoid)
- Carbon source (Fatty acids)
- PhaA
- Acetoacetyl-CoA
- PhaB
- (R)-3-Hydroxyacyl-CoA
- PhaC

**Pathway III**

- **Fatty acid biosynthesis**
- Maltopyranose
- Maltose
- Acetyl-CoA

In the first pathway, PHA biosynthesis occurs from carbohydrates unrelated in structure to the final PHA monomer (Kazunori et al., 2001). Three key enzymes are implicated: β-ketothiolase, NADPH-dependent acetoacetyl-CoA reductase, and PHA synthase. The β-ketothiolase catalyses the condensation of two acetyl-CoA molecules from the tricarboxylic acid cycle. The resulting acetoacetyl-CoA subunits are then converted to 3-hydroxybutyryl-CoA and the PHA synthase catalyses the esterification of these subunits leading to the formation of P(3HB) (Philip et al., 2007). This pathway can also be utilised for the synthesis of P(3HB-co-3HV).

The second pathway involves the production of PHAs via the fatty acid degradation pathway. In this case, the resulting monomers in the polymer chain were similar in structure to the carbon source or shortened by 2, 4 or 6 carbon atoms.
(Huisman et al., 1989). In this pathway the fatty acids are first converted to the corresponding acyl-CoA which are then oxidised by the β-oxidation pathway via enoyl-CoA, (S)-3-hydroxyacyl-CoA and 3-ketoacyl-CoA precursors. Finally, enzymes like the enoyl-CoA hydratase, hydroxyacyl-CoA epimerase, and β-ketoacyl-CoA reductase connect the β-oxidation pathway with the medium-chain length PHA biosynthesis, through the PHA synthase (Rehm et al., 2007).

The third pathway involves mcl-PHA production via the fatty acid de novo biosynthetic pathway. This pathway is of significant interest due to the ability of producing mcl-PHAs from carbohydrates that are structurally unrelated to the carbon source and are inexpensive (Kazunori et al., 2001). In this pathway, the carbohydrates are first oxidized to acetyl-CoA molecules that enter into the fatty acid de novo biosynthetic pathway. The fatty acid de novo biosynthesis leads to the formation of R-3-hydroxyacyl-ACP precursor, which is then linked to the PHA synthase for the mcl-PHA biosynthesis via the (R)-3-hydroxyacyl-ACP-CoA transacylase (Chen 2010).

PHAs producer microorganisms

More than 250 strains were described as PHAs producers, however, only a few of them are usually employed for PHA production. Among the most used are Cupriavidus nociator, Alcaligenes latus, Bacillus megaterium, Pseudomonas oleovorans and Pseudomonas putida, due to their capacity to growth in a range of substrates and their ability to synthesize a wide range of PHAs depending on the carbon source and the cultivation condition used (Chen et al., 2010). P(3HB) is the most common and widely studied PHA (Lemoigne et al., 1926). Since then, various bacterial strains among Gram positive and Gram negative bacteria have been identified to accumulate P(3HB) both aerobically and anaerobically. Awareness of the cost of production of PHAs and need of industrialization encouraged several groups to work intensively in reducing the production costs. In 1990, Hangii reported A. latus as the main candidate for P(3HB) production due to the fast growth and the ability to use cheap carbon sources (Hangi 1990). However, since then, many different strains and strategies have been developed for the P(3HB) production. Yu et al. achieved a 57% P(3HB) dry cell weight(dcw) yield under appropriate C/N ratios in C. necator (Yu et al., 2008). Omar et al. reported P(3HB) content of cells of up to 50% when B. megaterium was fed with date syrup and beet molasses (Omar et al., 2001). Akaraonye et al. obtained 67% dcw yield of P(3HB) with Bacillus cereus SPV in the presence of sugarcane molasses (Akaraonye et al., 2011).

Among the mcl-PHA producer microorganisms P. oleovorans was the first bacteria reported to produce an mcl-PHA copolymer containing P(3HO) when grown on n-octane as the sole carbon source (De Smet et al., 1983). After this finding, Haywood et al. examined various Pseudomonas species for growth and polymer accumulation with different alkanes, alcohols and alkanolic acids as the sole carbon source and proved that mcl-PHA production were not only restricted to P. oleovorans but also to P. aeruginosa, P. putida, P. fluorescens, and P. testosterone (Haywood et al., 1989). Later, it was found that mcl-PHAs accumulated mainly by Pseudomonas belonging to rRNA-DNA homology group I and that a number of strains in this group were able to produce scl- and mcl-PHA copolymer (Kabilan et al., 2012). Mcl-PHAs and copolymers attracted a lot of attention due to their flexible and elastomeric properties for industrial and particularly biomedical applications where flexible biocompatible biomaterials are required. Liu et al produced Poly(3-hydroxydecanoate-co-dodecanoate), P(3HD-co-3HDD), copolymer when P. putida was grown on dodecanolic acid as a single carbon source. Mechanical characterization of the polymer properties showed the flexible nature of
the material (Liu et al., 2011). Rai et al., reported the production of an absolute homopolymer of P(3HO) when P. mendocina CH50 was growth in octanoate in contrast to other well studied organisms such as P. putida, P. oleovorans, P. aeruginosa, P. resinovorans and P. stutzeri which accumulate copolymers. Mechanical, thermal, and chemical analysis carried out on the P(3HO) homopolymer revealed the flexible, elastomeric and semicrystalline structure of the material (Rai et al., 2011).

**Recombinant PHA producer microorganisms**

One of the most popular strategies for enhancing the type, quality or quantity of the produced PHAs consists in the homologous or heterologous expression of the PHA biosynthetic enzymes in different microorganisms including PHA or non-PHA producers. Several used strategies aim to reduce the cost of the polymers by developing recombinant strains able to utilize cheap carbon sources and free of PHA degradative pathways to accumulate high amounts of PHAs. For example, Park et al over expressed the PHA biosynthetic genes in C. necator showing increased levels of P(3HB) and reduced fermentation times (Park et al., 1997). Povolo et al. converted C. necator, a strain unable to grow on lactose, into an organism capable of growing on lactose contained in waste material by cloning the Escherichia coli lac genes along with the PHA depolymerase gene. Higher PHA yield was obtained compared to the wild type strain (Povolo et al. 2010).

Other strategies have focused on the development of novel PHAs by introducing new PHA biosynthetic pathways or genes into different strains. Li et al. cloned PHA biosynthetic genes from Aeromonas caviae into P. putida allowing the recombinant bacteria to produce a scl-mcl-PHA copolymer consisting of P(3HB), Poly-3-hydroxyvalerate P(3HV) and Poly-3-hydroxyheptanoate P(3HHp). The resulting copolymer was shown to have highest tensile strength and stiffness compared with other commercially available PHAs (Li et al.). Ma et al. showed that when the 3-hydroxyacyl-CoA dehydrogenase gene was partially or completely deleted in P. putida, the produced copolymer composed of P(44% 3HD-co-3HDD) showed the highest melting temperature and Young’s modulus among all the studied PHAs (Ma et al., 2009).

Escherichia coli is one of the most widely used hosts for the production of heterologous PHAs as it has shown outstanding results in standard recombinant expression applications and a capacity for large scale production to meet commercial demands (Sorensen et al., 2005). Zheng et al. worked on the development of an E.coli capable of synthesising PHAs and succinate from a mixture of glycerol, glucose and fatty acids (by-products of the biodiesel production process) by overexpressing the phaC1 gene from P. aeruginosa. The resulting strain was able to synthesize succinate and a copolymer composed of 3HO and 3HD (Zhenget al., 2011). Presence of toxic lipopolysaccharides present in all Gram-negative strains, which are co-purified with PHAs limited the use of these polymers in medical applications.

Singh et al. described Bacillus subtilis as a potential host for the production of PHAs. Gram-positive bacteria lack LPS and hence they are preferred hosts for the production of PHAs for biomedical applications (Valappil et al., 2007). In particular,

B. subtilis is generally recognized as a safe (GRAS) organism by the Food and Drug administration (FDA) and is among the most studied and widely used microbes for large-scale production of recombinant proteins, amino acids and chemicals. In addition, B. subtilis subsp. subtilis was described as a non-PHA producer like E. coli, and hence it can also be used for the expression and study of PHA biosynthetic
genes (Singh et al., 2009). Wang et al. have worked on the expression of the phaC1 gene from Pseudomonas aeruginosa in B. subtilis DB104, in the presence of glucose as a carbon source. Results showed that the recombinant bacteria were able to produce P(3HD-co-3HDD). Furthermore, the incorporation of the phaA gene, encoding the β-ketothiolase and the phaB gene encoding the acetoacetyl-CoA-reductase from R. eutropha resulted in the production of a Poly(3-hydroxybutyrate-co-3-hydroxydecanoate-co-3-hydroxydodecanoate P(3HB-co-3HD-co-3HDD), when malt waste was used as carbon source (Wang et al., 2006).

Production of PHAs by fermentation

PHA production in bioreactors was carried out in batch, fed-batch and continuous processes (Jung et al., 2001, Sun et al., 2007, Suwannasing et al., 2011). In a batch process, the bioreactor is supplied with fresh media and inoculum. At the end of the fermentation process, the content of the bioreactor is harvested and the polymer is extracted. In fed-batch processes, fresh substrate is continuously supplied in the bioreactor during the fermentation process. In this case, cells are allowed to grow exponentially until stationary phase. At this point, specific substrates were added to promote the production of specific PHAs. In some cases, when cells grow exponentially and nutrients start to be consumed, specific carbon sources are supplied to allow an increment in the cell concentration, the production phase and the final product. Continuous processes are based on a continuous feed and withdrawal of nutrients and culture from the system. In this case, a continuous flow is maintained with the substrate concentration maintained at one desirable level, to achieve a high cell concentration, followed by a product formation phase with nutrient depletion (Rehm et al., 2009).

Based on the culture conditions required for the PHA synthesis, bacteria can be divided into two major groups. One group requires an excess supply of carbon and limitation of nitrogen, phosphorus, oxygen or magnesium such as C. necator and P. oleovorans, while the second group do not require nutrient limitation for PHA accumulation such as A. latus, A. vinelandii and recombinant E. coli. These characteristics are important to be considered for deciding the fermentation strategy for PHA production. The fermentation condition should be designed to allow cells to grow to a high density for high productivity and then to stop growing or dividing and redirect their metabolism to the accumulation of PHAs (Jurasak et al., 2004). Generally, when processes involve a complete depletion of a nutrient, fed-batch fermentations were utilized with PHA accumulation occurring during the nutrient depletion stage (Kim et al. 1997; Lee et al. 2000; Diniz et al. 2004).

As different strains require different growth conditions, the fermentation strategy used for PHA accumulation varies with the organism used. Additionally, physiological conditions used have a direct effect in PHA subunit composition, cellular PHA content, specific PHA synthesis rate and overall volumetric productivity (Sun et al., 2007). Knop et al. reported that when A. vinelandii was grown in batch culture, under an oxygen limiting condition, a reduction in the activity of the tricarboxylic acid cycle, and the redirection of acetyl-CoA molecules resulted in P(3HB) production (Knop et al., 1989). Later, Page et al. reported higher yield of PHAs when A. vinelandii was growth in fed-batch cultures, with high aeration during the first stage and low aeration in the second stage, prompting P(3HB) formation (Page et al., 1997). Presutig et al. studied mcl-PHA production using P. oleovorans showing that the specific mcl-PHA accumulation rate is strongly dependent on the specific growth rate of the strain and is highest when it grows at 0.2 h⁻¹, which is less than half of the maximum specific growth rate (Presutig et al., 1991). Jung et al. have worked on the production of mcl-PHAs in two-stage
continuous cultivation with a dilution rate of 0.2 and 0.16 h\(^{-1}\) in the first and second stage. Under these conditions, P. oleovorans cells contained 63% dcw PHAs, which was one of the highest PHA yield obtained in P. oleovorans (Jung et al., 2001). Hence, as optimal conditions for PHA production are not the same in all cases, it is necessary to assess the optimal condition for different bacteria, carbon source or media composition employed.

One of the main limitations in the PHA production is the production cost. For example, it has been reported that PHA production is 10 times more expensive than polyethylene production (Kasemsap et al., 2007). The most important limitation factor in the production of PHAs are the special growth conditions required, the media utilized, the fermentation process and the PHA recovery. Hence, several groups have focused in developing systems that allow a high volumetric productivity. This parameter will define the size of a product needed to meet market demands. Currently, one of the main PHAs produced at industrial scale, is the copolymer P(3HB-co-3HV) by C. necator, due to the cost effectiveness of the process (Verlinden, 2007).

It is well known that trying to reproduce results obtained in shaken flask, in bioreactors is a difficult task and in many cases the variables involved are not very well understood. For this reason, there is a need to find the optimal growth conditions when bioreactors are used (Peña et al., 2011). Once the optimal growth condition for a specific strain and media composition is achieved, PHA production can be scaled-up. Scale-up studies based on the constant power input or constant oxygen transfer parameters have been carried out. The constant power input scaling-up criteria is based on a constant amount of energy required to maintain fluid motion within a vessel, in a given period of time. Power input is often referred to as volumetric power consumption and is representative of the turbulence degree and media circulation in vessels, and influences heat and mass transfer, mixing and circulation times (Marques et al., 2010). On the other hand, the constant oxygen transfer technique is based in keeping the same oxygen transfer rate (OTR) at different scales. The dissolved oxygen concentration in a suspension depends on the rate of oxygen transfer from the gas phase to the liquid phase, the rate at which oxygen is transported into the cells and on the microorganism oxygen uptake rate. In stirred tank bioreactors, different variables affect the mass transfer, however, the main ones are the stirrer speed, type and number of stirrers and gas flow rate used. Hence, the correct measurement of the OTR is a crucial step for the prediction of the conditions for larger scale production (Garcia-Ochoa et al., 2009).

In summary, Polyhydroxyalkanoates are a well-researched family of polymers which have excellent potential as biomaterials for a large range of applications.

2.1.2 Poly(lactic acid) (PLA)

Lactic acid exists in several distinct chiral forms. Polylactide or polylactic acid (PLA) is the product of lactide polymerization. Figure 3\(^1\) shows the cycle in which lactic acid is produced from plant resources, to lactide formation, over ring polymerization of the PLA through the polymer degradation seeing lactid acid metabolized to carbondioxide and water.

Reports dating back as far as the 18th century describe the self-condensation of lactic acid to yield solid materials. The polymer was not really recognized until the Carothers working group reported lactide polymerization in 1932. PLA is biodegradable under certain conditions and many PLA-based film applications are proven to be industrially compostable according to the recognized standard EN 13432, bearing the compostability certifications of TÜV Austria (OK Compost, formerly owned by Belgian certifier Vinçotte) or DIN CERTCO (DIN geprüft industrial compostable). It is also fully bio-based as it is derived from renewable resources such as corn or sugarcane. PLA is, therefore, suitable for innovative applications in which biodegradability is a desirable feature (e.g. biowaste collection bags), and it can help to reduce the reliance on finite fossil resources in the production of plastics. In the 1970s, the advantages of biodegradable polyesters were recognized, and the potential that PLA has shown in novel biomedical applications, such as tissue engineering and wound healing, indicate that it will be an important material for future high value medical markets.

PLA can be produced starting with hydrolysed starch or sucrose transformed to lactic acid (2-hydroxy propionic acid) building blocks, which exist in optically active D- or L-enantiomers. Depending on the proportion of the enantiomers, PLA with variable properties can be derived. This enables the development of different processing methods for the production of a wide range of PLA grades with suitable properties. In the past, the usage of PLA has been mainly limited to biomedical areas due to its biodegradability and biocompatibility characteristics. The discovery of new polymerization methods, which allow the economical production of PLA with high molecular weight have resulted in an expanded use of PLA for consumer goods, packaging applications, medical applications such as

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implant devices, tissue scaffolds\textsuperscript{4}, and internal sutures, and pharmaceutical applications\textsuperscript{5}. Its low toxicity, along with sustainability aspects connected to its renewability and biodegradability, have made PLA an ideal material for the food packaging and consumer product industries\textsuperscript{6}. According to the leading PLA producer, NatureWorks LLC, PLA is now being manufactured on a 140,000 ton scale annually in the USA and on a smaller scale by several companies in Europe and Japan\textsuperscript{7}.

The PLA building block, lactic acid, can be produced by carbohydrate fermentation or chemical synthesis. The majority of lactic acid production is based on the fermentation route. Different purification technologies for lactic acid and lactide are described in a recent review by Datta and Henry\textsuperscript{8}.

The high molecular weight PLA (greater than 100,000 g mol\textsuperscript{-1}) is one of the main drivers for the economical production and consequent expanded use of this polymer in recent times. There are two methods to prepare poly(lactide): the condensation of lactic acid and the ring opening polymerization of lactide, Figure 4\textsuperscript{9}.

![Figure 4 Synthesis of poly(L-lactide) from L-lactic acid](image)

The condensation polymerisation is impeded by low molecular mass, including the hindrance to reaching very high conversions in order to access polymers with sufficient molecular weight to have useful strength. As esterification reactions are equilibrium processes, it is necessary to remove the water in order to achieve high conversions. Although direct condensation of lactic acid should be the cheapest route of PLA production, the need for coupling agents adds to the cost and makes this reaction economically unprofitable. Therefore, high molecular weight commercial PLA is produced via the ring-opening polymerization route\textsuperscript{10}\textsuperscript{11}. Lactide, the cyclic dimer formed by condensation of lactic

\textsuperscript{6} C. J. Weber, V. Haugaard, R. Festersen, G. Bertelsen, Production and applications of biobased packaging materials for the food industry, Food Addit Contam 19 (2002) 172–177
\textsuperscript{8} http://www.natureworksllc.com/
acid, is a useful building block for poly(lactide) production as it is easily purified. The ring opening polymerization usually initiated by metal complexes, organic compounds, or enzymes yield poly(lactide) with high molecular weight, with the enthalpy of polymerisation as driving force. This enables us to overcome adverse polymerization entropy12. Controlled polymerisations are desirable due to the ability to predict and properly adjust the properties of the polymer through the quantity and the nature of the initiator used. Initiator biocompatibility/toxicity are also important considerations for application in skin-contact or medical and pharmaceutical products. Biocompatibility is especially difficult to assess for initiators systems. Alkoxides and Sn(II) are generally considered non-toxic and suitable for polylactide preparation. Their hydroxides are strong bases, which can be irritant or harmful, and therefore, under certain conditions, the formation of the hydroxides would be undesirable. The organocatalytic initiating systems are not toxic and therefore are suitable for polylactide applications, which require the absence of any metal contamination.

Lactide polymerization in the presence of tin octanoate, Sn(Oct)₂ as initiator has been studied for more than twenty years. During this time, several mechanisms have been proposed including the cationic, activated monomer, and the coordination-insertion mechanism typical of other metal alkoxides13,14. Sn(Oct)₂ was known to be contaminated by water and octanoic acid, which were difficult to remove15. The intermediate product, in lactide polymerisation, undergoes acyl-oxygen bond cleavage of the lactide ring and generates a new metal alkoxide and a growing polymer chain with an ester capped end group. From the late 1980s, when Kricheldorf used organic acids to polymerise lactide, the use of different organic molecules as initiator for lactide polymerisation by cationic mechanism has been known, although with poor polymerization control16. Two groups used triflic acid (or its ester) as initiator for lactide polymerization (and glycolide). Triflic acid acts both as initiator and as catalyst and reacts with lactide forming a linear unimer terminated at one end with a hydroxyl group and at the other end with mixed anhydride group (figure 517). Triflic acid also protonates monomeric lactide and propagation proceeds by the activation monomer mechanism as reaction of protonated lactide molecules with terminal hydroxyl groups.

Poly(lactide) is a biocompatible material and can be designed to biodegrade within a reasonable timescale, which make this polymer useful for various biomedical and environmental applications. For controlled release systems, polylactide can be used as a matrix or microspheres carrier. Pharmaceutical substances can be released from the polymer matrix, with the controlled release rate depending on the polymer structure. The biomaterials synthesised for drug delivery must allow fine-tuning of the final properties and the adjustment of release kinetics and the degradation time.

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However, the release of active substances from a drug is controlled by erosion of the bulk material and by the outpouring. Biodegradable microspheres prepared from poly(lactide) have been widely studied in recent years and have become well established controlled drug delivery systems\(^\text{18 19}\). Poly(D,L-lactide) microspheres are mainly used for drugs which can cause undesirable effects if used in higher quantities, or in case of a drug’s insolubility. With the incorporation in microspheres such deficiencies of drugs can be diminished by enabling safe handling for longer periods of time through the release of optimal quantities of drugs\(^\text{20 21}\). Moreover, microspheres can be used for locally targeted drug delivery, which is a general prerequisite for drug release supposed to take place only at the diseased location instead of throughout the body\(^\text{22 23}\).

![Lactide polymerization](image)

**Figure 5** Propagation of lactide polymerization according to the activation monomer mechanism

Biodegradable polymers are often used as matrices for active substances, primarily due to their low toxicity. They also provide controlled release of the drug at the site of action during a certain period of time. Drug nanoencapsulation increases the efficiency of and tolerance for the drug and represents a superior alternative to conventional drug taking\(^\text{24}\). Important properties of polymeric nanoparticles as drug carriers are particle size and particle size distribution, surface morphology, charge and adhesion, surface erosion, diffusion, encapsulation efficiency, stability, drug release kinetics and

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\(^{18}\) J. Herrmann, R. J. Bodmeier, Control Release 36 (1995) 63
\(^{21}\) A. B. M. Kumar, K. P. Rao, Biomaterials 19 (1998) 725
hemodynamics (Feng, 2004). In order to meet an increasing demand in this field and to achieve better therapeutic efficiency of drugs, significant efforts are invested into developing new and improving existing biodegradable polymers (Ristic et al., 2011; Ristic et al., 2013). Biodegradable polymers used in medicine and pharmacy should be biocompatible and their degradation products should be non-toxic.

The ideal biodegradable polymer for use in medicine and pharmacy should have the following key properties: it does not cause inflammations or toxic reactions; it can easily be sterilised; after serving its purpose, it can be enzymatically biodegraded by an appropriate metabolic pathway and eliminated from the organism (Ristic, 2011).

PLA fulfils these requirements and therefore is a natural choice for the applications targeted in PolyBioSkin. There are various different commercial PLA grades available. The differences are based on molecular weight and content of D-lactic acid units. The molecular weight affects the rheological properties (viscosity in the melt) significantly, whereas the content of D-lactic acid units affects thermo-mechanical properties. As an example, Ingeo 2003D PLA of NatureWorks is a PLA grade for general extrusion, whereas their Ingeo 6060D PLA is indicated for the production of fibres.

PLA is used for large-scale industrial applications in the packaging sector, as the operation of big synthesis plants has resulted in significant price reductions. In fact, currently the cost of PLA is similar to that of major conventional petrochemical commodity plastics such as polystyrene (PS). The preparation of materials based on PLA for large-scale applications, such as the ones targeted in PolyBioSkin, can be considered economically acceptable to the market.

PLA-based materials are successfully used in packaging applications, because they have properties that allow the substitution of conventional hydrocarbon-based packaging. PLA can be processed in the 150–200°C temperature range by injection moulding, sheet extrusion, blow moulding, thermoforming, and film forming, but the obtained material is usually stiff and brittle. The improvement of mechanical properties is needed for this material to be used in applications currently reserved to polyolefins. For this purpose, both the addition of plasticizers and blending with other biodegradable polymers are explored in PolyBioSkin. The most successful blends can be expected to be prepared through blending with PBAT and PBS.
2.1.2.1 Poly(lactic-co-glycolic acid) (PLGA)

Poly(lactic-co-glycolic acid) is a copolymer of poly(lactic acid) and poly(glycolic acid), with a varying ratio of glycolic acid (from 10% to 50%), which has a direct influence on the degradation rate. PLGA exhibits a faster degradation rate than PLA and is therefore suitable for use when faster delivery of active compounds is needed. Its most common application is in drug delivery systems, because of the great release profile. It is very suitable for pharmaceutical and medical applications since the products of its degradation are lactic acid and glycolic acid, which are not harmful to the human organism.

2.1.3 Poly(butylene adipate-co-terephthalate) (PBAT)

Poly(butylene adipate-co-terephthalate) (PBAT) is a polyester produced by polycondensation of 1,4-butandiol (BDO), terephthalic acid (TA) and adipic acid (AA). When its production was first started by BASF, all the component monomers were obtained from non-renewable resources. In successive years, the efforts of large companies (such as Coca-Cola) to produce bio-PET led to the commissioning of large production facilities producing TA. US-company BioAmber and Italian company Novamont both have announced to be able to produce bio-based BDO. The US-based company Verdezyne developed facilities for the production of renewable AA, so that today both acids can be produced from renewable sources on a small but significant industrial scale. It is reported that a minimum amount of 35% of AA is required in the acid components to achieve the full biodegradability of PBAT. The current commercial products of BASF have a content of AA not defined in the technical sheets. However, since the product is certified industrially compostable by independent certification bodies, it can be hypothesized that the content of AA is between 35% and 55%. Following this approach, and considering the molecular weight of the different repeating units, it is possible to calculate that the current content of BDO is about 35%. PBAT in the market is predominately fossil-based, but in some cases bio-based BDO is already used. Based on the current research results, however, it is expected that within only a few years, fully renewable (100%) PBAT will be commercially available on an industrial scale.

![Figure 6 Chemical structure of PBAT](image.png)

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The blending of PBAT and PLA leads to a biphasic blend, as PBAT is immiscible with PLA. The blending with PBAT allowed the preparation of materials with interesting mechanical properties, such as a good level of toughness\textsuperscript{41}.

2.1.4 Poly(butylene succinate) (PBS)

Poly(butylene succinate) (PBS) is an aliphatic, biodegradable polyester. It is synthesized by polycondensation reaction of 1,4 butanediol (BDO) and succinic acid (SA)\textsuperscript{42} and has the molecular structure shown in figure 7. At the beginning, as in the case of PBAT, PBS was biodegradable but petro-based. The Bionolle brand polymers of Showa have been commercially available for more than a decade ago. Currently, large plants for producing renewably sourced SA were developed and Mitsubishi commercializes bio-PBS that is obtained by using biobased SA. Furthermore bio-based BDO can be produced at industrial scale (see PBAT), thus 100% renewable PBS is in the market, offered by companies such as e.g. Succinity\textsuperscript{43}.

![Figure 7 Chemical structure of PBS](image)

PBS is of particular interest because of its mechanical properties. It is a crystalline polymer with a glass transition temperature of about -35 °C and a melting point around 114 °C. Hence, at room temperature it behaves as a ductile polymer and may exhibit elongation-to-break in excess of 300%, depending on molecular weight. Melt blending of PBS with PLA would appear to be an ideal route for improving properties of these polymers because of their complementarity: increasing the toughness of PLA and the stiffness of PBS.

It was found that the polymer was more ductile if during the polymerization of SA and BDO also some adipic acid (AA) or lactic acid (LA) was added. Hence various commercial products are available on the market containing a few percent of these two acids. In the case AA is copolymerized the copolymer is called PBSA, and where lactic acid was copolymerized the copolymer is called PBSL. The commercially available bio-PBS can thus exhibit different properties because of different molecular weights and also because of different co-monomer content.

2.2 Polysaccharides

2.2.1 Pullulan

Pullulan is a water-soluble, neutral, and linear glucosic polysaccharide, which is produced by fermentation of liquefied cornstarch by the polymorphic fungus \textit{Aureobasidium pullulans}. It is used in

\textsuperscript{41} Jiang, L.; Wolcott, M. P.; Zhang, J. Biomacromolecules 2006, 7, 199.
\textsuperscript{43} For more information visit http://www.succinity.com.
a variety of applications, for example, as plasma substitute and food additive, as adhesive or film-forming agent, and as cosmetic thickener or carrier ingredient. The polymer is composed of repeating maltotriose units joined by α-1,6-linkages. The glucose units within the maltotriose monomer are connected by α-1,4-glycosidic bonds. The commercial product has a molecular weight from 100,000 to 200,000 Daltons.

Commercial pullulan is classified as food ingredient in Japan and in the USA has the status of a GRAS (Generally Recognised as Safe) ingredient, which is also used as excipient in pharmaceutical tablets. This sugar-like polymer shows distinctive physical properties such as adhesive ability, capacity to form fibres and thin biodegradable films, which dissolve rapidly in water, are transparent and impermeable to oxygen, and oil and grease resistant. Moreover, as an edible, non-toxic and non-carcinogenic polymer, it possesses a very interesting releasing capability and immunomodulatory abilities. Finally, being able to quench free radicals thanks to its anti-oxidant properties, pullulan protects mesenchymal cells from oxidative damage. This is important, for example, after a cutaneous injury, where the initial phase of wound healing is characterized by a massive influx of inflammatory cells that secrete abundant reactive oxygen species (ROS) to kill bacteria and defend the disrupted skin barrier. However, since the excess ROS can be detrimental to wound repair leading to cell damage and impaired response to injury, it is necessary to manage the oxidative stress, rebalancing both ROS and natural anti-oxidant secretion through the use of ingredients such as pullulan, nano-chitin, and nano-lignin as important components of innovative non-woven tissues. Pullulan has therefore been selected as one the target materials to be used in electrospun non-woven tissues for the production of a skin-contact carrier film, specifically in a dry facial beauty masks, which has the added benefit over wet masks to avoid the use of preservatives.

2.2.2 Starch

The polysaccharide starch is an assimilation product of green plant cells. The molecule deposits as small, colourless starch granules in the cell chloroplasts. The starch granules usually consist of about 80% amylopectin and about 20% amylose. However, the ratio of both polymers depends on the plant type: some corn types can contain more than 98% amylopectin. Amylose consists predominantly of unbranched chains of glucose molecules (AGUs) which are linked by α-1,4-glycosidic bonds (α-1,4-glucon). Amylose builds up as a spiral helix with 6 D-glucose units per winding. The relative molecular weight varies from 17000 to 225000, which equals a sequence of 100 to 1400 glucose units within the chain.
Amylopectin is the main component of starch and consist also of D-glucose units. Though the glucose units are arranged in bush-like branched and shorter chains, containing 20-25 glucose units each. The bonds within the chain are α-1,4 glycosidic, but α-1,6- glycosidic bond at the branching points. The relative molecular weight of amylopectin is about 200000 to 1000000.

The most common raw materials for starch are potatoes, grain, corn or rice. Starch is a colourless, hygroscopic powder, which is insoluble in cold water. Heating up water to about 90°C starch paste is formed, due to swelling of the amylopectin. In opposite amylose is dispersed in colloids.

Starch is an important polymer for the food and the paper industry, but is more and more used also for textiles or in plastic applications. To get mouldable starch, also known as thermoplastic starch, the granules are destructured by extrusion. For this a certain amount of mechanical energy and heat is necessary as well as addition of plasticisers. The structure, including the helix is destroyed during this process. Due to the hydrophilicity and brittleness TPS, is usually used in blends (together with PBAT or PLA) and can be processed by film blowing, injection moulding and blow moulding.

Owing to its complete biodegradability, low cost and renewability, starch is a promising candidate for developing sustainable materials and therefore nonwovens. The medical use of starch-based polymers as explored for example the fabrication of scaffolds for bone-tissue engineering because of their biocompatibility and porous nature, which allows blood-vessel proliferation during bone growth.

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44 Figure from: https://chemistry.stackexchange.com/questions/58080/bonding-between-amylopectin-and-amylose (accessed January 2018)
Due to the many hydroxyl groups on its chains, starch can easily interact with other polar polymers through the formation of hydrogen bonds and can be also oxidized or reduced to modify its structure and functionalities. As a consequence, it is possible to improve the final physicochemical properties of composites made with other natural polymers. At this purpose for example, to improve the compatibility between the hydrophilic starch and the hydrophobic PLA or PHAs suitable plasticizers are often used. On the other hand, the starch/chitin blend exhibits good film forming properties due to the intermolecular hydrogen bonding, formed between the amino and amide groups on chitin and the hydroxyl groups on the backbone of starch.\(^49\)

### 2.2.3 Cellulose

Cellulose is the most abundant polymer on earth, which means that it also the most common organic compound on the planet. The yield of cellulose via photosynthesis is estimated around 830 million metric tons per annum. As 40% of dry-weight of crops is composed of cellulose and its annual output is approximately 200 million tons\(^50\). Various natural fibres such as cotton and higher plants have cellulose as their main constituent. Plants contain approximately 33% cellulose whereas wood contains around 50% and cotton contains 90%. Cellulose is a linear and fairly rigid homopolymer consisting of D-anhydroglucopyranose units (AGU) with each cellulose molecule having three hydroxyl groups per AGU, with the exception of the terminal ends. These AGU units are linked together by β-(1–4) glycosidic bonds formed between C-1 and C-4 of adjacent glucose moieties (Figure 10).

![Chemical structure of cellulose](image)

Figure 10 Chemical structure of cellulose\(^50\).

Cellulose is insoluble in water and most common solvents; the poor solubility is attributed primarily to the strong intramolecular and intermolecular hydrogen bonding between the individual chains. Since plant polysaccharides comply with many requirements expected of pharmaceutical excipients such as non-toxicity, stability, availability, and renewability, they are extensively investigated for use in the development of solid oral dosage forms. Various forms of cellulose have many other permitted FDA uses, including as a fat substitute and bulking agent in low calorie foods, as a texturizer, emulsifier, and extender.

Obtained as a pulp from fibrous materials such as wood or cotton, although it was used in pharmaceutical applications such as a filler in tablets, most of the Cellulose is extensively used as a raw material by the paper industry for the production of paper and cardboard products and a small fraction is used in the production of commodity materials and value added cellulose derivatives (cellulosics). Cellulosics are in general strong, reproducible, recyclable and biocompatible, being used in various biomedical applications such as blood purification membranes and the like. Among all these modified cellulosic products, carboxymethyl cellulose (CMC) is manufactured in significant amounts due to its wide commercial applications with regard to volume demand\(^51\).

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\(^{49}\) This is taken from an article by Morganti, Danti, Coltelli, entitled “Biobased tissues for innovative cosmetic: PolyBioSkin as an EU research project”, in print, 2018.


\(^{51}\) Haleem et al. (2014) “Synthesis of carboxymethyl cellulose from waste of cotton ginning industry”. Carbohyd Polym (113) 249-255.
2.2.3.1 Carboxymethyl cellulose (CMC)

Sodium carboxymethyl cellulose (CMC) (Figure 11) is the most widely used cellulose ether today with applications in various industries. It is a man modified water-soluble cellulose derivative that is produced by the 2-step reaction of cellulose with alkali and monochloroacetic acid (MCA), using organic solvents, under heterogeneous conditions. In the first step, the cellulose is treated with NaOH in the presence of an inert solvent, which acts both as a swelling agent and as a diluent and thus facilitates good penetration of NaOH into the cellulose structure. The alkali cellulose is accessible and reactive towards MCA, which is added to the reaction in the second step. The CMC can be neutralized and dried immediately to achieve a technical grade, or neutralized and washed to provide a purified grade. Purified grades for industrial applications (detergents, adhesives, pesticide, lubricants, cloth, cement, ceramics, oil drilling mud, and in the paper and coating industry) often have a purity of about at least 98%, while CMC grades for food, pharmaceuticals and personal care applications have a purity of at least 99.5%.

![Figure 11 Chemical structure of carboxymethyl cellulose](image)

The properties of cellulose derivatives are mainly determined by the degree of substitution (DS), viscosity, and particle size. CMC is soluble in water when the DS is higher than 0.5. The theoretical maximum DS that can be achieved is 3, due to three for modification accessible hydroxyl groups. The selection of carboxymethylation conditions is essential in order to prepare highly substituted CMC. In commercial CMC, the most common DS obtained is usually lower, ranging from 0.4 to 1.4. Researchers are trying to develop alternative ways of increasing the degree of substitution for CMC in order to produce better commercial products. However, increasing the DS may reduce the biodegradability.

CMC appears as a good precursor for bio-SAP synthesis in the PolyBioSkin project, because of its biocompatibility, its bio-based origin (from wood), and the rigidity of the cyclic cellulose backbone polymer, which provides very high levels of superabsorbency when CMC is crosslinked.

2.2.4 Chitin

Chitin, poly(β-(1→4)-N-acetyl-d-glucosamine), is one of the most important polysaccharides in the world. It occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. Depending on the source, chitin occurs as three allomorphs, namely the α, β, and γ forms. In both α and β-structures, the chitin chains are organized in sheets where they are tightly held by a number of intra-sheet hydrogen bonds. This

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tight network, dominated by the rather strong C–O···HN hydrogen bonds, maintains the chains very close to each other along the parameter of the unit cell (Figure 12). In α-chitin, there are also some inter-sheet hydrogen bonds along the b axis of the unit cell, involving an association of the hydroxymethyl groups of adjacent chains.

Figure 12 1) α-chitin molecular structure and hydrogen bonding in alpha-chitin. The coloured circles represent the different hydrogen bonding inside the chitin crystal structure. 2) Structure of α-chitin: (a) ac projection; (b) bc projection; (c) ab projection. The structure contains a statistical mixture of 2 conformations of the –CH2OH groups.

Chitin production

Worldwide production of chitin has been estimated to be around $10^{11}$ tons per annum produced from a marine fishery of ~150 billion tons/year, the discards of which exceed ~25% of the total annual production. The fishery by-products, in fact, are considered hazardous for their high perishability and high polluting effects if disposed into the sea.

By chitin alkaline hydrolysis with a degree of deacetylation over 60%, chitosan is obtained, a polymer consisting of ~80% of glucosamine and ~20% acetyl glucosamine units.

Effect of chitin and chitin nanofibrils on humans and the environment

Chitin is easily metabolized by environmental chitinases and human chitotriosidases, with a preference of acetylated (i.e. chitin) versus non-acetylated sugars (i.e. chitosan). Chitotriosidase, in fact, is involved in the innate immunity activation and polarization cascades of macrophages, as well as the indirect activation of other immune cells such as T-helper cells and eosinophils. In analogy to the function of homologous chitinases in plants, the physiological role of chitotriosidases is most likely

an innate immunity toward chitin containing pathogens\textsuperscript{56}. It is important to underline that the anti-inflammatory, immunomodulatory, and antibacterial effectiveness of chitin is strictly connected with its lower size dimension\textsuperscript{57}. These activities have been recently confirmed, showing that chitin nanofibrils have the ability to promote the release of the antibacterial defensins and modulate the activity of metalloproteinases\textsuperscript{58}. Thus, this sugar-like compound offers a protective and defensive activity to plants and humans, being also easily enzymatically biodegraded through metabolism into carbon dioxide and water with zero waste residue.

**Nano-Chitin processing technology**

Chitin nanofibrils (CNs) (Figure 13) are obtained from chitin through a technology patented by PolyBioSkin industrial consortium member MAVI, which removes their amorphous regions, preserving their high crystallinity domains. The result is pure crystals of a mean dimension of 240x7x5nm.

![Figure 13 Chitin Nanofibrils at SEM.](image)

CNs, obtained as a 2% water suspension (~3 billion/ML), have been characterized by FT-IR spectrum, TEM, SEM microscopy, and Dynamic Light Scattering, determining its physicochemical characteristics, the crystal mean dimension, and its mean molecular weight of $0.074.10^{6}$ \textsuperscript{59}.

Moreover, it has been shown that when positively charged with ~15.000 amino groups per particle, these crystals may be complexed by the gelation method with negatively charged polymers or macromolecules such as, for example, hyaluronan or lignin (Figure 14)\textsuperscript{60}. Moreover, during this process, it is possible to entrap single or multiple active ingredients characterised for their hydrophilic or hydrophobic functions. The obtained micro/nano particles may be poured into gels/emulsions or entrapped into non-woven tissue through the electrospinning technology\textsuperscript{61}.


Properties of chitin nanofibrils

The CNs properties depend on their crystalline purity and the electrical charges covering their surface. This polymer is made of repeating units of glucosamine and acetyl glucosamine, because during the production process a certain quantity of acetyl groups are hydrolysed with formation of glucosamine, not present in the native chitin. Thus CN, enriching its molecular backbone with free amino groups, acquires some of the chitosan’s properties. These pure crystals, therefore, possess the capacities of both glucosamine and acetyl glucosamine, natural molecules acting, for example, as protective molecules of the human bone during its movement. Moreover, the cationic activity of the glucosamine amino groups permits the formation of the complexes based on electrostatic forces, and a formation of intra- and inter-macromolecular C-O···HN bonds occurs\(^2\). CN, used as carrier, has also been shown to accelerate the skin cicatrising activity by avoiding the formation of hypertrophic scars and keloids\(^3\). In line with the end-of-life requirements of PolyBioSkin, composites containing chitin nano-fibrils were found to be readily biodegradable\(^4\).

Current uses of chitin

The main characteristics of chitin and chitosan are: high porosity, biodegradability, predictable degradation rate, structural integrity, non-toxicity to cells, and biocompatibility. Drawing on these remarkable characteristics, they are to-date used for a range of biomedical and cosmetic applications such as tissue engineering, as biomaterials for wound healing, drug delivery and cancer diagnostic, as antiseptic for ophthalmology, to produce designed nanocarriers and enable microencapsulation techniques, to fabricate polymer scaffolds, as base dressing and anti-aging agent for cosmetics. However, many of these uses are yet at an experimental level and there needs to be a push for an increased utilisation for large-scale industrial purposes.

There is a distinct opportunity to use CN as a carrier not only for innovative facial beauty masks, but as a driver for the marketization of a whole new category of cosmetic products made by biodegradable non-woven tissues.

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\(^4\) Patrizia Cinelli, Maria Beatrice Coltell, Norma Mallegni, Piefrancesco Morganti, Andrea Lazzeri, Degradability and Sustainability of Nanocomposites Based on Polylactic Acid and Chitin Nano Fibrils, Chemical Engineering Transactions, 60, 115-120 DOI: 10.3303/CET1760020
To date, the fast-growing multi-billion dollar global cosmetics market is still populated both by products containing active ingredients demonstrated to have a clinical effectiveness, and a host of make-believe recipes containing, for example, vegetable extracts from exotic countries’ exotic plants, food ingredients such as chocolate or caviar, or precious metals such as gold, pearl, or ruby, that, despite the lack of any scientific evidence for their efficacy, fire the imagination of consumers in their quest for eternal youth. To be attractive to consumers, cosmetic and skin-care products need to feature innovative formulations, be highly effective within a short time, must be of natural origin (or rather to be perceived as somehow in harmony with nature), and free of any side effects, ‘chemicals’, or harmful environmental impacts.

To meet these requirements and design commercially viable, sustainable and sustainably competitive products, a great deal of research and in vitro and in vivo studies of how selected ingredients work to improve the appearance of and protect the skin and help maintain its natural functions, in accordance with all applicable regulations for cosmetic products, is required.

The cosmetic effectiveness of active ingredients is due not only to their innate characteristics, but also to the appropriate release from the vehicle used, at the right skin layer, at the right dose, and at the designated time, according to the so called 4R’s. The efficacy and the selectivity of the formulation, therefore, depends to a very significant extent on the carrier used and the designs and complexations involved, which is why these aspects have become of primary importance to obtain in the bid to achieve the desired effects. Thus nanotechnology, the integration of engineering with biology, chemistry, and physics, and innovative processing technologies are starting to be employed in the cosmetic field to drive promising innovation. However, to produce an effective and stable cosmetic solution, gel, ointment, or emulsion, it is necessary to use emulsifiers, preservatives, and other chemicals, and often these ingredients become the cause of allergic or sensitising skin reactions.

The proposal of the PolyBioSkin project is to produce innovative and biodegradable non-woven tissues. They will be used for designing facial beauty mask sheets as a new category of cosmetic products that are more skin-friendly due to the absence of emulsifiers, preservatives, chemicals, and colours, and characterized in vitro and in vivo for their effectiveness and safeness. Moreover, they will have a greatly increased sustainability potential for being ≥90% bio-based and fully biodegradable both by human enzymes and by bacteria present in a range of environments, complying with the requirements of the EN 13432 standard, as will be demonstrated in later work packages.

2.2.5 Chitosan

As previously reported, chitosan is obtained through a deacetylation procedure from chitin so that the obtained primary amine groups can be conjugated with various kind of molecules. Consequently, many different derivatives have been developed, such as PEGylated chitosan or carboxymethyl cellulose to obtain water-soluble compounds. As opposed to chitin, chitosan is soluble in acidic water. Up to now chitosan is used in the medical and other fields much more than chitin, because of its good solubility\textsuperscript{66}. Only recently the use of chitin has increased because of the possibility to obtain its nanofibrils by an industrial methodology.

Various studies show that solutions of chitosan in concentrations ranging from 0.5% to 5% w/w in acidic water exhibit antimicrobial effects on a wide variety of microorganisms. The antimicrobial activity was demonstrated \textit{in vitro} on a wide range of microorganisms such as yeasts, bacteria\textsuperscript{67}, and molds\textsuperscript{68-69} with very heterogeneous endpoints; antimicrobial efficacy is influenced by intrinsic factors of the chitosan molecule\textsuperscript{70}, environmental factors, the types of microorganism concerned, and the physical state\textsuperscript{71} where chitosan is supposed to exert the biocidal function.

![Figure 16 Structure of Chitosan](image)

2.3 Other biopolymers

2.3.1 Lignin

Lignin\textsuperscript{72}, making up 10-25% of lignocellulosic biomass, is the second major component of wood and annually growing plants, and the most abundant natural polymer after cellulose and chitin. It is a three-dimensional, highly cross-linked macromolecule composed of three types of substituted phenols, which include coniferyl, synapyl, and p-coumaryl alcohols.


Derived from native lignin, industrial and technical lignins are available in large amounts as by-product of cellulose pulp in papermaking. This by-product contains highly branched, irregular phenolic macromolecules, the structure of which depends on botanic origin, harvesting period, and extraction process. Lignin, which is insoluble in water, acts as the glue that connects cellulose and hemi-cellulose. However, it is soluble in alkaline, releasing free phenolic monomers, mainly as p/coumarin acids.

Due to its aromatic structure and the occurrence of phenol residues, lignin has shown to have multiple potential activities, such as antimicrobial, antioxidant and photoprotective. Recently, different kinds of industrial lignin tested for their cytotoxicity have shown to be safe so that lignin-based products may be incorporated into cells. Moreover, being an electronegative macromolecule, lignin has been complexed with CN to obtain micro/nano particles embedded into emulsions and non-woven tissues that are skin-friendly (Figure 18).

So far, large quantities of lignocellulosic biomass are being used primarily as essential material source for biofuels, obtaining bioethanol and biobutanol. It is only recently that the interesting biological characteristics and the beneficial properties of lignin for human health have moved into the focus of scientific interest.

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Design of complexes containing active molecules and their potential applications in PolyBioSkin

Building on the expertise and experience of the PolyBioSkin industrial partner MAVI in the production and utilisation of complexes of chitin nanofibrils and lignin, it is possible to design complexes containing functional molecules entrapped between the positive chitin nanofibrils and the negative lignin nano-particles.

A range of such functional molecules has been pre-selected and the final selection will be introduced in later work packages. For the functionalisation of the diaper surface, antiseptic and anti-inflammatory complexations will be required, for the functionalization of facial beauty sheet masks, antioxidant formulations are indicated, and for wound dressings, antiseptic and anti-inflammatory properties are required.

2.3.2 Gelatin

Gelatin is a mixture of peptides and proteins, derived from different body parts of animals, which are usually obtained from wastes of the meat and leather industries. It can be produced by acid, alkali and enzymatic hydrolysis from collagen and the method of production defines the type of resulting gelatin. It is insoluble in cold water but swells and forms hydrogel. It can be dissolved in hot water and some other polar solvents. It can be crosslinked using aldehydes, commonly glutaraldehyde. Except in foodstuffs and for cooking, gelatin is widely used in the pharmaceutical industry for capsules and drug delivery systems.

2.3.3 Poly(ethylene glycol) (PEG)

Despite favorable structural stability and biocompatibility properties, chemically crosslinked hydrogels may present drawbacks, including the low water-solubility of polymer precursors (e.g., cellulose, chitosan) and intrinsic cytotoxicity of common chemical crosslinkers due to the risk of unreacted species in the system. Therefore, an alternative to overcoming the afore-mentioned disadvantages is to prepare hydrogels using chemically modified polymer derivatives such as CMC to promote its water-solubility and use biocompatible and eco-friendly crosslinkers (e.g., citric acid, polyethylene glycol) throughout the entire process.

Polyethylene glycol (PEG) is a polyether that is amphiphilic and soluble in water as well as in many organic solvents. PEG is readily available in a wide range of molecular weights, it has been found to be non-toxic, and is approved by the U.S. Food and Drug Administration (FDA). These features contribute to its broad application in biomedical research, drug delivery, tissue engineering scaffolds, surface functionalization, and so forth.
Polymers such as PEG (Figure 19) have been combined with cellulose and CMC systems as an effective network modifier to improve the properties of the hydrogels.\footnote{Capanema et al. (2018). “Superabsorbent crosslinked carboxymethyl cellulose-PEG hydrogels for potential wound dressing applications”, Int J Biolog Macromol (106) 1218-1234.}

PEG available in the market is mainly fossil-based, but options made from renewable resources are also available, e.g. by US company Acme-Hardesty, who offer partly bio-based PEGs made from bagasse with USDA certification.\footnote{http://www.biobased.polyethylene-glycol-peg-400/ (accessed January 2018)}

3 Commercial competitiveness of PolyBioSkin biopolymer materials and target applications

Very often the cost associated with the sourcing, production, processing, and disposal of bio-based polymers has been considered the Achilles heel of the bioplastics industry and a major obstacle to large-scale market introduction of these innovative sustainable materials. A holistic assessment of the cost competitiveness of bioplastics reveals a vicious circle in which the high initial opportunity cost of using biopolymers prevents the broader uptake of these materials, which in turn prevents the evolution of economies of scale that would be the biggest single factor in reducing prices. More recently, very low oil price levels have contributed significantly to bioplastic price premiums and stifled an otherwise sensible interest in diversifying the resource base for plastics in such a way as to include a significant bio-based share. Meanwhile, the concern over crude oil consumption for the production of conventional fossil-based polymers appears to be overshadowed by a public discourse about the detrimental effects of using agricultural feedstock for the production of bio-based Commodities, the so-called ‘land-use (change)’ and ‘food vs. feed’ debates. Nevertheless, bioplastic production is gaining in volume and market share.\footnote{For current and forecasted global bioplastic production capacity and market data visit http://www.european-bioplastics.org/news/publications/}

A key aspect of the resilient and fast growth of the bioplastics industry is the fact that beyond fossil-based plastic substitution potential (and the sustainability benefits associated with it), many biopolymers offer innovative new functionalities and enhanced performance in comparison to the fossil-based and non-biodegradable materials that we used to have. In comparison to simple applications such as plastic shopping bags which are extremely price sensitive, the cost factor may lose in significance against the performance enhancements in more complex high-end but nevertheless ubiquitous mass market applications such as the ones PolyBioSkin focuses on. Immense research and development efforts are underway to explore and realise the potential of bioplastics and to accelerate the progression from using virgin bio-based feedstocks to biogenic waste resources, increasing both the utility and the sustainability of bio-based plastics. With crude oil being a highly demanded but ultimately finite resource, these factors will progressively pave the way for greater price competitiveness.
Looking at the price levels for materials used in PolyBioSkin and the respective benchmark materials commonly used, such as polyurethane, polyethylene, or polypropylene, comparisons can be drawn on a basic raw material level. Wageningen UR Food & Biobased Research provides such data for a range of common (bio)plastics (see table 2 and 3)\textsuperscript{79}.

<table>
<thead>
<tr>
<th>Plastic</th>
<th>Price level 2016 (€/kg)</th>
<th>Density (kg/m\textsuperscript{3})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>5</td>
<td>1200 – 1300</td>
</tr>
<tr>
<td>Bio-PA</td>
<td>+ 10 – 20%</td>
<td>1040 – 1190</td>
</tr>
<tr>
<td>Bio-PE</td>
<td>+ 20 – 40%</td>
<td>910 – 970</td>
</tr>
<tr>
<td>Bio-PET</td>
<td>No information</td>
<td>1370 – 1390</td>
</tr>
<tr>
<td>Bio-PP\textsuperscript{4}</td>
<td>+ 80 – 100%</td>
<td>900 – 920</td>
</tr>
<tr>
<td>PP (certified bio)\textsuperscript{5}</td>
<td>+ 40 – 50%</td>
<td>900 – 920</td>
</tr>
<tr>
<td>PBAT</td>
<td>3.5</td>
<td>1250</td>
</tr>
<tr>
<td>Bio-PBS</td>
<td>4</td>
<td>1260</td>
</tr>
<tr>
<td>PHA</td>
<td>5</td>
<td>1200 – 1250</td>
</tr>
<tr>
<td>PLA</td>
<td>2</td>
<td>1250</td>
</tr>
<tr>
<td>PTT</td>
<td>4</td>
<td>1320</td>
</tr>
<tr>
<td>Starch blends</td>
<td>2 – 4</td>
<td>1250 – 1350</td>
</tr>
</tbody>
</table>

Table 2 Price level for bio-based and/or biodegradable plastics in 2016 based on WFBR experience

Drop-ins such as bio-based PE are already achieving fairly competitive price levels, and PLA, importantly, comes very close to the prices levels of high-volume commodity plastics such as PET and polystyrene, depending to a large extent on the given prices of feedstock, i.e. oil on the one hand, and sugar on the other hand.

The same cannot be said for important biodegradable materials such as PBAT or PHAs. That said, there is no direct comparability, as biodegradable plastic materials offer new functionalities, rendering them suitable for a whole new range of applications where they can realise distinctive performance improvements. In addition, there are other aspects that can remedy a simple weight-based price disadvantage of bioplastics. For example, the Wageningen Report cites material savings that can be realised when using PLA thanks to its higher stiffness in comparison to conventional commodity plastics such as polystyrene. Cost savings like this are perceived only when analyses are performed at the product level rather than a raw material level. Similarly, if labour cost is taken into account when calculating the overall cost of using a particular type of product, functionalities such as biodegradability can realise significant cost savings. A good example of this is the use of biodegradable mulch films in agriculture, where the use of conventional polyethylene mulch films would require significant labour and other expenditures for the subsequent removal and recycling of films from fields.

These are the aspects that PolyBioSkin seeks to exploit. The peculiar features of the novel materials introduced in this report, such as biodegradability and an enhanced level of biocompatibility are crucial for designing better products, and better performing products can reduce overall cost levels.

<table>
<thead>
<tr>
<th>Plastic</th>
<th>Price level 2016 (€/tonne)</th>
<th>Density (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDPE</td>
<td>1250 – 1450</td>
<td>910 – 940</td>
</tr>
<tr>
<td>HDPE</td>
<td>1200 – 1500</td>
<td>930 – 970</td>
</tr>
<tr>
<td>HIPS</td>
<td>1350 - 1525</td>
<td>1080</td>
</tr>
<tr>
<td>PET</td>
<td>850 – 1050</td>
<td>1370 – 1390</td>
</tr>
<tr>
<td>PP</td>
<td>1000 – 1200</td>
<td>900 – 920</td>
</tr>
<tr>
<td>PS</td>
<td>1250 – 1430</td>
<td>1040</td>
</tr>
<tr>
<td>PVC</td>
<td>800 – 930</td>
<td>1100 – 1450</td>
</tr>
</tbody>
</table>

Table 3 Price level of fossil-based plastics in 2016 (Vraag en aanbod, 2016).

Looking at the PolyBioSkin target applications, this is especially true for biomedical wounds dressings, where shorter healing times result directly in lower expenditures associated with medical staff labour costs and generally with the stationary care of in-patients. Procurers of advanced wound dressings for medical institutions will not hesitate to pay premium prices for premium products, and performance is key to successful marketisation.

In a similar vein, the commercial success of facial beauty masks will not depend to a very significant extent on the cost of manufacture. The market for facial beauty masks is growing at a rapid pace, especially in the Asia-Pacific (APAC) region where further large retail market value increases are projected over the next years, with China (29% increase), India (23%), Indonesia (19%), Malaysia (41%), Japan, and South Korea (both 16%) featuring the highest growth figures in the 2017-2019 period. Within the facial beauty mask market, sheet masks have the largest market share, and their market alone is currently estimated at $160.4 million (2016). With a predicted CAGR of 8.7%, this revenue is expected to grow to a staggering US$336.7 million by 2024. The global facial beauty sheet mask market is segmented into four major fabric materials used to produce them: non-wovens, hydrogel, bio-cellulose, and cotton. Of these, the bio-cellulose fabric masks are expected to show the most remarkable growth during the forecast period.

The prices for sheet masks (and other types of beauty masks) differ greatly. Based on some of the spotlight launches in 2016 and 2017 covered in the Mintel Report, consumers may be paying anything between US$36 for an 8-pack of H₂O + Beauty’s Waterbright Water-Infused Brightening Gel Mask, US$9 for a single sheet of the LuxaDerme Brightening Bio Cellulose Sheet Mask or EUR€9.40 for 1

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80 Murphy and Evans (2012), p. 4, estimate that „advanced dressings typically are changed every 1 to 3 days, as opposed to gauze, which is often changed multiple times per day”

81 SpotLight on Facial Masks, Mintel Report, 2017, p. 7


83 https://www.h2oplus.com/waterbright-water-infused-brightening-gel-mask

sheet of the Starskin Red Carpet Ready Hydrating Bio Cellulose Second Skin Face Mask\textsuperscript{85}, to GBP£18 for a single one of Charlotte Tilbury’s Instant Magic Facial Dry Sheet Masks\textsuperscript{86}. There are off-brand sheet mask products widely available for less than or in the region of €1 per mask, for example the Balea or Schaebens ranges of German drugstore DM\textsuperscript{87}.

The pricing of beauty masks is not necessarily a function of their performance, i.e. the extent to which claims made in favour of a product may or may not be backed up by evidence of actual efficacy. In the field of cosmetics there is not much in the way of regulation that would require manufacturers to provide evidence supporting claims such as the ones customarily made in connection with beauty masks, for example: anti-ageing, moisturising/hydrating, reduction of fine lines and wrinkles, whitening, pore refining, etc. Buying choices in this market appear to be determined to a large extent by marketing techniques and consumers’ subjective perceptions of effects such as, for example, sensations of refreshment or cooling.

With that in mind, it can be assumed that the PolyBioSkin beauty masks, rather than being at a disadvantage for relying on biomaterials, would on the contrary benefit from the fact that they offer a sustainability-improved product – justifying a “green premium” – that will be demonstrated to have the beauty and well-being effects it claims to have, thanks to the novel technologies and formulations employed in their production.

In the case of the targeted \textit{absorbent hygiene products} – diapers and femcare sanitary pads – the situation is more complex and challenging. These types of products are indispensable to a great number of people around the globe and, for many of them, pricing naturally becomes a very sensitive issue. Nevertheless, performance improvements, for example, with regards to anti-inflammatory properties, and the ‘green premium’ are expected to create a commercial niche for these products especially with environmentally conscious consumers who already pay premium prices for a range of goods. The consortium aims to achieve a production cost level initially around one-and-a-half to two times the level of conventional commercial products.

On the macroeconomic rather than individual consumer level, the PolyBioSkin AHP target applications aim to offer an additional value proposition: AHPs, and especially used baby diapers, constitute an enormous amount of post-consumer waste, which is either being incinerated or worse landfilled, with one estimate saying that AHPs account for around 1.5% - 6.5% of landfilled waste in Europe\textsuperscript{88}. The development of biodegradable diapers will fuel on-going efforts to develop significantly more sustainable end-of-life scenarios specifically for large volume applications that are contaminated after use and thus not allowed into existing mechanical or organic recycling streams, for example the BBI-funded EMBRACED project which realises a biorefinery based on the valorisation of AHP waste\textsuperscript{89}. In a way, AHP waste is too valuable to let it go to waste, and with its focus on bio-based and biodegradable materials and solutions, PolyBioSkin aims to put innovative materials to competitive advantage.

\textsuperscript{86} http://www.charlottetilbury.com/uk/dry-sheet-face-mask.html
\textsuperscript{87} https://www.dm.de/pflege-und-duft/gesichtspflege/serum-masken-und-peelings/
\textsuperscript{89} For more information, visit https://bbi-europe.eu/projects/embraced.
The expectation of commercial competitiveness of bio-based and biodegradable applications like the ones pursued in PolyBioSkin thus rests on three pillars:

- Being based on renewable feedstocks, sourced from annually re-growing plants and/or even organic waste, rather than finite crude oil sources, these developments follow an inherently logical economic rationale, namely that fossil-based materials will eventually and in the long-term experience price hikes. Feedstock diversification makes sense even today as a way of reducing the risks of price shocks in one’s supply chain.
- Secondly, many biopolymers have valuable qualities that conventional materials lack. Harnessing the biodegradability, biocompatibility, anti-inflammatory, anti-oxidant, and anti-septic capabilities of the materials introduced above and further ingredients that will be employed in the project constitutes a value proposition with significant exploitation potential.
- Lastly, the drive towards increased sustainability can be turned into commercial advantage on an individual level and is beneficial for society at large.

4 Summary and outlook

A range of suitable biopolymers has been pre-selected and introduced in this report based on the technical expertise and knowledge of the academic and industrial consortium partners, and no fundamental barriers to commercial exploitation have been identified.

In subsequent tasks and work packages (WPs), the specific formulations for the required films, fibres, and tissues will be determined based on this pre-selection (WP 1-4), and the various components will be engineered for prototyping and testing (WP 5-7). WP 8 will provide a comprehensive assessment of sustainability, life cycle, biodegradation, and health and safety considerations, as well as detail standardisation and regulatory aspects. In addition, dissemination and exploitation strategies will be formulated and executed in WP9 to ensure a high level of project result uptake.